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SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Gailene R. Gabel Examiner #: 76197 Date: 8/11/01
Art Unit: ~~1637~~ 1641 Phone Number 30 5-0807 Serial Number: 09/555 102
Mail Box and Bldg/Room Location: 7B15 Results Format Preferred (circle): PAPER DISK E-MAIL
7412 CM1

If more than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Multiple Assay Method

Inventors (please provide full names): Thomas, Nicholas

Earliest Priority Filing Date: 6/10/98

***For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.**

(1) "bead?
"particle?
"microsphere?

population?
Subpopulation?
Subset?
group

(near prox.)

distinguish?
distinct?
part?
separate?

Flow Cytomet?

compound?
toxin?
test material?
drug?
pharmaceutical?
analyte?

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CM1 12C14 Tel: 308-4994

Please search highlighted terms. Thanks.
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Date Searcher Picked Up: _____
Date Completed: 08-16-01
Searcher Prep & Review Time: 12
Clerical Prep Time: _____
Online Time: 49

Type of Search

NA Sequence (#) _____
AA Sequence (#) _____
Structure (#) _____
Bibliographic _____
Litigation _____
Fulltext _____
Patent Family _____
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Vendors and cost where applicable

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Dialog _____
Questel/Orbit _____
Dr.Link _____
Lexis/Nexis _____
Sequence Systems _____
WWW/Internet _____
Other (specify) _____

PTO-1590 (1-2000)

Gabel
09/555102

FILE 'CAPLUS' ENTERED AT 11:19:22 ON 16 AUG 2001

L1 3296 SEA FILE=CAPLUS ABB=ON PLU=ON N(W)(SAMPL? OR SPECIMEN OR POPULAT? OR SUBPOPULAT? OR GROUP OR SUBSET)

L2 208 SEA FILE=CAPLUS ABB=ON PLU=ON L1 AND (?BEAD? OR ?PARTICLE? OR ?SPHERE? OR ?SPHERICAL?)

L3 1 SEA FILE=CAPLUS ABB=ON PLU=ON L2 AND (FLOW(W)(CYTOMETER? OR CYTOMETR?))

L4 3049471 SEA FILE=CAPLUS ABB=ON PLU=ON (SAMPL? OR SPECIMEN OR POPULAT? OR SUBPOPULAT? OR GROUP OR SUBSET)

L11 222045 SEA FILE=CAPLUS ABB=ON PLU=ON L4(5A)(ASSAY? OR DETERM? OR DET## OR SCREEN? OR DETECT? OR DISTING? OR SEPARAT? OR SORT?)

L12 13294 SEA FILE=CAPLUS ABB=ON PLU=ON L11 AND (?BEAD? OR ?PARTICLE? OR ?SPHERE? OR ?SPHERICAL?)

L13 93 SEA FILE=CAPLUS ABB=ON PLU=ON L12 AND (FLOW(W)(CYTOMETER? OR CYTOMETR?))

L14 27 SEA FILE=CAPLUS ABB=ON PLU=ON L13 AND (COMPOUND OR COMP## OR TOXIN OR DRUG OR PHARMACEUT? OR ANALYTE OR TEST(W)MATERIAL)

L15 18 SEA FILE=CAPLUS ABB=ON PLU=ON L14 AND (LABEL? OR DYE? OR STAIN? OR PRECOAT? OR COAT? OR REAGENT)

L16 18 L3 OR L15

L16 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2001:526322 CAPLUS

TITLE: Multiplex flow assays preferably with magnetic **particles** as solid phase

INVENTOR(S): Watkins, Michael I.; Edwards, Richard B.

PATENT ASSIGNEE(S): USA

SOURCE: U.S. Pat. Appl. Publ., 13 pp.
CODEN: USXXCO

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2001008217	A1	20010719	US 1999-302920	19990430

AB Heterogeneous assays for different **analytes** in a single biol. sample are performed simultaneously in a multiplexed assay that combines **flow cytometry** with the use of magnetic **particles** as the solid phase and yields an individual result for each **analyte**. The **particles**

are distinguishable from each other by characteristics that permit them to be differentiated into **groups**, each **group** carrying an **assay reagent** bonded to the **particle** surface that is distinct from the **assay reagents** of **particles** in other **groups**. The magnetic **particles** facilitate sepn. of the solid and liq. phases, permitting the assays to be performed by automated equipment. Assays are also disclosed for the simultaneous detection of antibodies of different classes and a common antigen specificity or of a common class and different antigen specificities. Each type is accomplished by immunol. binding at the surfaces of two distinct solid phases in a sequential manner with dissocn. of the binding and washing of the solid phase in between the binding steps. Three types of magnetic **beads coated** with antigens of cytomegalovirus, herpes simplex virus 2, and rubella virus were reacted with patient samples in a simultaneous multi-**analyte** immunoassay. The **beads** were washed and the liq. phase was removed before the **beads** were further reacted with anti-human IgG-phycoerythrin conjugate. The samples were then injected into a **flow cytometer**.

L16 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 2000:874154 CAPLUS
DOCUMENT NUMBER: 134:16515
TITLE: Evaluation of autoimmune diseases using a multiple parameter latex **bead** suspension and **flow cytometry**
INVENTOR(S): Hechinger, Mark K.
PATENT ASSIGNEE(S): Affinitech, Ltd, USA
SOURCE: U.S., 45 pp., Cont.-in-part of U.S. Ser. No. 404,144, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6159748	A	20001212	US 1998-88648	19980601

PRIORITY APPLN. INFO.: US 1995-404144 B2 19950313

AB The author discloses immunoassay methods which utilize **flow cytometry**, **coated** latex **microspheres**, and **labeled** antibodies, to **detect** antibodies in a **sample**. The **microspheres** can be sized by forward angle light scatter (FALS) or electronic vol. and, by combining FALS and fluorescence, it is practical to use **beads** of several different sizes for the simultaneous

detection of multiple **analytes**. In one example, the ribonucleoproteins Sm, SS-A, SS-B and Scl-70 were bound to 4, 5, 6, 7 and 10 .mu.m latex **beads**, resp. and stabilized for extended shelf-life. Dild. serum was placed into test tubes contg. a mixt. of the five antigen **coated beads** and incubated. After washing the **bead**/serum mixt., a second incubation with goat anti-human IgG, conjugated with a fluorochrome, is carried out. The fluorescence intensity is based on the avidity of the **bead**/antibody/conjugate binding.

REFERENCE COUNT: 7

REFERENCE(S):
(1) Anon; GB 1561042 1980
(2) Bonfa; New England Journal of Medicine 1987, V317(5), P265 MEDLINE
(3) Elkon; The Journal of Immunology 1985, V134(6), P3819 CAPLUS
(4) Fisk; US 3088875 1963 CAPLUS
(5) Fulwyler; Methods in Cell Biology, Chapter 51 1990, V33, P613 MEDLINE
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 3 OF 18 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 2000:326995 CAPLUS

TITLE: Simultaneous analysis of protein, bacterial, and viral antigens using a **flow cytometric** microarray immunosensor.

AUTHOR(S): Venkateswaran, Kodumudi S.; Langlois, Richard G.

CORPORATE SOURCE: Biology and]Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA, 94551, USA

SOURCE: Book of Abstracts, 219th ACS National Meeting, San Francisco, CA, March 26-30, 2000 (2000), ANYL-201. American Chemical Society: Washington, D. C.
CODEN: 69CLAC

DOCUMENT TYPE: Conference; Meeting Abstract

LANGUAGE: English

AB Multiple **analyte detection** in a **sample** reduces the cost and time of the anal. Only few immunosensor formats are available for anal. of more than one **analyte** at the same time. We report here the development of a **flow cytometric** microarray immunosensor for concurrent detection and identification of protein, bacterial and viral antigens. Polystyrene **spheres** with distinct fluorescence properties were used as solid support for this flow **microsphere** immunoassay. Antigen-specific capture antibodies were covalently coupled to each set of optically encoded fluorescent **microspheres**. The test sample contg. the **analytes** was incubated with a mixt. of **microspheres** followed by

analyte-specific reporter fluorescent-**labeled** antibodies. Multi-parametric **flow cytometric** anal. can distinguish each set of **microspheres** based on two different classifying fluorescence emission. Simultaneous measurement of the reporter fluorescence on the **microspheres** can reveal the presence or absence of **analyte**. This microarray immunosensor was used to simultaneously detect four simulants of biol. agents - protein **toxin** (Ovalbumin, Ov), bacillus spore (Bacillus globigii, Bg), vegetative bacteria (Erwinia herbicola, Eh) and bacteriophage MS2. **Flow cytometric** multiplex assay could detect concns. ranging over four orders of magnitude and was comparable to conventional single **analyte** immunoassay. This assay can be performed in a microtiter plate format in less than an hour. Hence **flow cytometric** microarray immunosensor is useful for large scale multiplexed immuno detection.

L16 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:796055 CAPLUS
DOCUMENT NUMBER: 132:20824
TITLE: Multiple assay method
INVENTOR(S): Thomas, Nicholas
PATENT ASSIGNEE(S): Amersham Pharmacia Biotech UK Limited, UK
SOURCE: PCT Int. Appl., 26 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9964867	A1	19991216	WO 1998-GB3727	19981203
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 1036332	A1	20000920	EP 1998-959060	19981203
R: CH, DE, ES, FR, GB, IT, LI, NL, SE				

PRIORITY APPLN. INFO.: EP 1997-309784 A 19971204
WO 1998-GB3727 W 19981203

AB A method for the **assay** of **N samples** each contg. a **compd.** to be tested, comprises providing N reaction vessels each contg. a population of carrier **beads** and other **reagents** for performing the assay, where N is at least 2 e.g. 80-4000. Each **population** of carrier **beads** is **distinguishable** from every other **population**. After adding the samples to the reaction vessels and performing the assays, the contents of all the reaction

vessels are mixed and subjected to anal. by **flow cytometry**. By means of **flow cytometry**, each carrier **bead** is rapidly analyzed to identify its **population** and also to **det**. the presence or concn. or biol. activity of the **compd**. to be tested.

REFERENCE COUNT: 7

REFERENCE(S):
(1) Affymax Tech NV; WO 9306121 A 1993 CAPLUS
(2) Luminex Corp; WO 9714028 A 1997 CAPLUS
(3) Mandecki Wlodek; US 5641634 A 1997 CAPLUS
(4) Nitto Denko Corp; JP 06027112 A 1994 CAPLUS
(5) Operon Technologies Inc; WO 9513538 A 1995 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 5 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:529341 CAPLUS
DOCUMENT NUMBER: 131:155517
TITLE: Methods and **reagents** for the rapid and efficient isolation of circulating cancer cells using immunomagnetic enrichment combined with **flow cytometric** and immunocytochemical analysis
INVENTOR(S): Terstappen, Leon W. M. M.; Rao, Galla Chandra; Uhr, Jonathan W.; Racila, Emilian V.; Liberti, Paul A.
PATENT ASSIGNEE(S): Immunivest, USA; University of Texas Southwestern Medical Center/Dallas
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9941613	A1	19990819	WO 1999-US3073	19990212
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9927636	A1	19990830	AU 1999-27636	19990212
BR 9907852	A	20001024	BR 1999-7852	19990212

EP 1062515 A1 20001227 EP 1999-908132 19990212
R: DE, FR, GB, IT, NL
PRIORITY APPLN. INFO.: US 1998-74535 P 19980212
US 1998-110202 P 19981130
US 1998-110279 P 19981130
WO 1999-US3073 W 19990212

AB A highly sensitive assay is disclosed which combines immunomagnetic enrichment with multiparameter **flow cytometric** and immunocytochem. anal. to detect, enumerate and characterize carcinoma cells in the blood. The assay can detect one epithelial cell or less in 1 mL of blood and has a greater sensitivity than conventional PCR or immunohistochem. by 1-2 orders of magnitude. In addn., the assay facilitates the biol. characterization and staging of carcinoma cells. Levels of circulating epithelial cells were **detd.** in peripheral blood **samples** from breast, prostate, and colon cancer patients and in normal controls. Blood was treated with anti-epithelial cell adhesion mol. (EpCAM) monoclonal antibodies coupled to magnetic **nanoparticles** and magnetically sepd. The collected fraction was treated with FACS permeabilization soln., magnetically sepd., and treated with phycoerythrin conjugated anti-cytokeratin monoclonal antibody and peridinin chlorophyll protein-**labeled** CD45. Magnetically sepd. material was further treated with a nucleic acid **dye**. The samples were analyzed by FACS **flow cytometry**.

REFERENCE COUNT: 7
REFERENCE(S):
(1) Berois, N; Anticancer Research 1997, V17(4A), P2639 CAPLUS
(2) Liberti; US 5512332 A 1996 CAPLUS
(3) Liberti; US 5597531 A 1997 CAPLUS
(4) Liberti; US 5698271 A 1997 CAPLUS
(5) Makarovskiy, A; Journal of Clinical Laboratory Analysis 1997, V11, P346 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:487468 CAPLUS
DOCUMENT NUMBER: 131:127388
TITLE: Detection system using liposomes and signal modification
INVENTOR(S): Nicklin, Stephen; Clarke, David John; Lloyd, Christopher James; Aojula, Harmesh Singh; Tsilosani, Marina; Wilson, Michael Thomas
PATENT ASSIGNEE(S): The Secretary of State for Defence, UK
SOURCE: PCT Int. Appl., 111 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9938009	A1	19990729	WO 1999-GB208	19990121
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9921770	A1	19990809	AU 1999-21770	19990121
EP 1049932	A1	20001108	EP 1999-901770	19990121
R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, LU, NL, SE, MC, PT, IE, FI				
NO 2000003709	A	20000921	NO 2000-3709	20000719

PRIORITY APPLN. INFO.: GB 1998-1120 A 19980121
WO 1999-GB208 W 19990121

AB A process for detecting an **analyte** comprises (a) contacting a sample suspected of contg. said **analyte** with a containment means comprising a barrier which **separates** signal generating **reagents** from said **sample**, in the presence of an element which interacts specifically with said **analyte**, under conditions whereby interaction between the **analyte** and the said element results in activation of the signal generating **reagents** within the containment means on the side of the barrier opposite to the **sample**, and (b) **detecting** any signal generated and retained within the containment means from the sample side of the barrier. The process of the invention provides for sensitive detection of very small nos. of **analyte** materials using measurement techniques which include counting methods such as **flow cytometry**.
TNT was detected using Tris-HCl pH 7.4, TNP-conjugated melittin as pore-forming **reagent**, liposomes contg. alk. phosphatase, ELF-97 substrate, and monoclonal antibodies to TNT. Fluorescent liposomes were counted.

REFERENCE COUNT: 7
REFERENCE(S):
(1) Anon; 1996, 3, CAPLUS
(2) Anon; 1998, 20, CAPLUS
(3) Eastman Kodak Company; EP 0255089 A 1988 CAPLUS
(4) Mizoguchi, H; JOURNAL OF FERMENTATION AND BIOENGINEERING 1996, V81(5), P406 CAPLUS
(6) Tutkimuskeskus, V; WO 9800714 A 1998 CAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1999:286112 CAPLUS
DOCUMENT NUMBER: 130:308788
TITLE: **Analyte** assay using scattered-light-detectable particulate **labels**
INVENTOR(S): Yguerabide, Juan; Yguerabide, Evangelina E.; Kohne, David E.; Jackson, Jeffrey T.
PATENT ASSIGNEE(S): Genicon Sciences Corporation, USA
SOURCE: PCT Int. Appl., 336 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9920789	A1	19990429	WO 1998-US23160	19981016
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CN 1223692	A	19990721	CN 1997-195868	19970417
AU 9912943	A1	19990510	AU 1999-12943	19981016
EP 1023456	A1	20000802	EP 1998-956415	19981016
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-953713 A 19971017
WO 1998-US23160 W 19981016

AB A method for specific detection of one or more **analytes** in a sample uses particulate **labels**. The method includes specifically assocg. any one or more **analytes** in the **sample** with a scattered-light-**detectable** **particle**, illuminating any **particle** assocd. with the **analytes** with light under conditions which produce scattered light from the **particle** and in which light scattered from one or more **particles** can be detected by a human eye with less than 500 times magnification and without electronic amplification. The method also includes detecting the light scattered by any such **particles** under those conditions as a measure of the presence of the **analytes**.

Poly(I) and poly(C) were activated with CDI and **labeled** with activated PEG-amine-**coated** 40 nm-diam. gold **particles**. The hybridization properties of the nucleic acid gold **particle** conjugates were studied using a light microscope.

REFERENCE COUNT: 6
REFERENCE(S):
(1) Drmanac; US 5202231 A 1993 CAPLUS
(2) Elghanian; Science 1997, P1078 CAPLUS
(4) Schutt; US 5017009 A 1991 CAPLUS
(5) Shalon; Genome Research 1996, P639 CAPLUS
(6) Stimpson; US 5599668 A 1997 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L16 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:470898 CAPLUS
DOCUMENT NUMBER: 129:259454
TITLE: Immunomagnetic separation and **flow cytometry** for rapid detection of Escherichia coli O157:H7
AUTHOR(S): Seo, K. H.; Brackett, R. E.; Frank, J. F.; Hilliard, S.
CORPORATE SOURCE: Center for Food Safety and Quality Enhancement, Food Science & Technology, University of Georgia, Experiment Station, Griffin, GA, 30223-1797, USA
SOURCE: J. Food Prot. (1998), 61(7), 812-816
CODEN: JFPRDR; ISSN: 0362-028X
PUBLISHER: International Association of Milk, Food and Environmental Sanitarians
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A rapid method for detecting Escherichia coli O157:H7 combining immunomagnetic **beads** (IMB) and **flow cytometry** was developed. **Labeling** antigens sepd. by IMB with fluorescent antibody enabled the detection of <103 CFU bacteria per mL in pure culture. The optimum concn. of magnetic **beads** for **flow cytometry** was lower (ca. 105 **particles** per mL) than that reported for conventional IMB assay (more than 6 .times. 106 to 8 .times. 106 **particles** per mL). Immunomagnetic sepn. and **flow cytometry** (IMFC) were evaluated for detecting E. coli O157:H7 in the presence of a competing microorganism and for detecting antibodies in potassium phosphate buffer. The total assay time from sepg. antigens with IMB to analyzing with **flow cytometry** was about 1 h. IMFC detected 103 to 104 CFU of E. coli O157:H7 per mL in ground beef enrichment broth and could effectively discriminate between E. coli O157:H7 and competing natural flora. The new assay system provides another approach to

sepn. and **detection** of low **populations** of pathogens and shows potential for detecting low concns. of **toxins** and other sol. antigens directly from food in a short time.

L16 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:384293 CAPLUS
DOCUMENT NUMBER: 129:104395
TITLE: The effect of 3-week tamoxifen treatment on estrogen receptor levels in primary breast tumors: a **flow cytometric** study
AUTHOR(S): Brotherick, I.; Browell, D. A.; Shenton, B. K.; Egan, M.; Cunliffe, W. J.; Webb, L. A.; Lunt, L. G.; Young, J. R.; Higgs, M. J.
CORPORATE SOURCE: Department of Surgery, University of Newcastle Upon Tyne, Newcastle Upon Tyne, NE2 4HH, UK
SOURCE: Br. J. Cancer (1998), 77(10), 1657-1660
CODEN: BJCAAI; ISSN: 0007-0920
PUBLISHER: Churchill Livingstone
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The effect of 3-wk, preoperative tamoxifen treatment on estrogen receptor (ER) levels, expressed by primary breast tumors, was examd. Patients (age-matched) with breast cancer, confirmed by fine-needle aspiration, were either treated with 20 mg ml-1 oral tamoxifen per day or received no medication in the 3-wk interval between assessment and surgery. Quantification of ER using **flow cytometry** was performed on the surgically removed tumor samples from tamoxifen-treated (n = 40) and control (n = 38, untreated) patient groups. The tumors were mech. disaggregated, and saponin treatment rendered these cells permeable to antibodies. Using dual-parameter **labeling** with a FITC-conjugated antibody (NCL-5D3) directed against cytokeratin 8/18/19 and a biotinylated antibody (DAKO-ER 1D5) directed against the estrogen receptor, ER quantification was detd. on a no. of receptors per cell basis. Using QC quantum **bead** stds., ER levels in the epithelial cell population, the non-epithelial cell population and the whole-cell population (ER +) were calcd. ER levels were significantly lower in the total cell population than tamoxifen-treated patients (P = 0.002) when compared with the control (untreated) group. By using a gating procedure using 5D3 antibody positivity, a significantly lower level was **detected** on examg. the cytokeratin-pos. **population** alone (P = 0.006). Using a complementary gating technique, ER levels were quantified in the cytokeratin-neg. cell population. Examn. of this group of cells showed no significant difference between the levels of estrogen receptor found in the

tamoxifen-treated and untreated groups (P = 0.4). We have demonstrated that ER levels can be monitored by **flow cytometry**. ER levels in patients treated with tamoxifen 3 wk before operation are significantly lower than in a comparative group of patients who received no **drug**. Furthermore, the most significant difference in receptor levels is seen by quantification of total ER levels expressed by all the tissue.

L16 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:746172 CAPLUS
DOCUMENT NUMBER: 128:11619
TITLE: Rapid assay for infection in neonates
INVENTOR(S): Weirich, Erica E.; Rabin, Ronald L.; Maldonado, Yvonne; Herzenberg, Leonore A.; Herzenberg, Leonard A.
PATENT ASSIGNEE(S): Board of Trustees of the Leland Stanford Junior University, USA
SOURCE: PCT Int. Appl., 37 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9742341	A1	19971113	WO 1997-US7781	19970507
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6077665	A	20000620	US 1997-833677	19970408
CA 2250911	AA	19971113	CA 1997-2250911	19970507
AU 9731191	A1	19971126	AU 1997-31191	19970507
EP 912756	A1	19990506	EP 1997-926421	19970507
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1996-17030 P 19960507
US 1997-833677 A 19970408
WO 1997-US7781 W 19970507

AB A rapid assay for infection in immunodeficient patients such as neonates or immunocompromised patients (e.g., HIV or transplant patients) is used in diagnosis at initial evaluation, such that antibiotic treatment and confinement to an intensive care unit are avoided for uninfected patients. The assay can be used for detecting bacterial, viral, or fungal colonization of the blood stream, cerebrospinal fluid, or urinary tract. The method is particularly suited for sepsis diagnosis. Polymorphonuclear leukocyte (PMN, neutrophil) CD11b (Mac-1, CR3) levels are measured

by **flow cytometry** or laser scanning microscopy in low vol. (0.1-mL) whole-blood samples. A dual-laser FACS identifies neutrophils by FITC-conjugated anti-CD15 fluorescent antibodies and identifies surface neutrophil CD11b marked with PE-conjugated anti-CD11b antibodies. Spontaneous upregulation of CD11b is prevented by handling samples at 4.degree. or adding a stabilizing **compd.** such as anti-CD14 antibody or adenosine to the samples. A kit comprises calibration markers such as CD11b-**coated microspheres** for calibrating the device used for anal. according to diagnosis threshold(s), as well as fluorescent anti-CD11b and anti-CD15 antibodies.

L16 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:640830 CAPLUS
DOCUMENT NUMBER: 127:302672
TITLE: Fluorescent reporter **beads** for fluid analysis
INVENTOR(S): Van Den Engh, Ger; Weigl, Bernhard H.
PATENT ASSIGNEE(S): University of Washington, USA
SOURCE: PCT Int. Appl., 39 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9735189	A1	19970925	WO 1997-US4099	19970314
W: AU, CA, JP				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5747349	A	19980505	US 1996-621170	19960320
AU 9740001	A1	19971010	AU 1997-40001	19970314

PRIORITY APPLN. INFO.: US 1996-621170 19960320
WO 1997-US4099 19970314

AB The present invention provides a method and app. for rapid measurements of a fluid bulk **analyte**, requiring only microscale vols. Several fluid bulk **analytes** can be measured simultaneously and, for biol. samples, the cell content can also be measured simultaneously. The invention comprises reporter **beads** for chem. anal. of fluid bulk properties such as pH, oxygen satn. and ion content. Each reporter **bead** comprises a substrate **bead** having a plurality of at least one type of fluorescent reporter mols. immobilized thereon. The fluorescent properties of the reporter **bead** are sensitive to a corresponding **analyte**. Reporter **beads** are added to a fluid **sample** and the **analyte** concn.

is **detd.** by measuring fluorescence of individual **beads**, for example in a **flow cytometer**. Alternatively, reporter mols. which change absorbance as a function of **analyte** concn. can be employed.

L16 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:391532 CAPLUS
DOCUMENT NUMBER: 122:155575
TITLE: Flow **microsphere** immunoassay for the quantitative and simultaneous detection of multiple soluble **analytes**
AUTHOR(S): McHugh, Thomas M.
CORPORATE SOURCE: San Francisco Medical Center, University California, San Francisco, CA, 94143, USA
SOURCE: Methods Cell Biol. (1994), 42(Flow Cytometry (2nd Ed.), Pt. B), 575-95
CODEN: MCBLAG; ISSN: 0091-679X
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Flow cytometer** requirements, **microspheres**, passive **coating**, and **sample** protocols for **assay** performance are discussed.

L16 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1995:321036 CAPLUS
DOCUMENT NUMBER: 122:100975
TITLE: Dual **analyte** assay based on **particle** types of different size measured by **flow cytometry**
AUTHOR(S): Frengen, Jomar; Lindmo, Tore; Paus, Elisabeth; Schmid, Ruth; Nustad, Kjell
CORPORATE SOURCE: Department of Physics, University of Trondheim, NTH, Trondheim, N-7034, Norway
SOURCE: J. Immunol. Methods (1995), 178(1), 141-51
CODEN: JIMMBG; ISSN: 0022-1759
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Simultaneous **flow cytometric** assays have been developed for .alpha.-fetoprotein (AFP) and human chorionic gonadotropin (hCG), with internal **detn.** of **sample** related nonspecific binding (NSB). The assays use **particles** of 7.5, 6.5 and 5.5 .mu.m diam. **coated** with, resp., monoclonal antibodies specific for AFP, hCG or an epitope normally not present in serum. The different **particle** types were identified simultaneously by light-scatter measurements as their specific immunofluorometric responses were detd. The NSB in the simultaneous assay of AFP and hCG was increased by .apprx.30% compared to corresponding single **analyte** assays. The

working range of the dual **analyte** assays was 0.6-2000 kIU/L for AFP and 6-10 000 IU/L for hCG. No significant interference from the presence of the other **analyte** was obsd. in the measurement of either AFP or hCG. The 95% confidence interval for the ratio of dual over single **analyte** assay results was [0.81, 1.11] for AFP and [0.88, 1.16] for hCG.

L16 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1994:650621 CAPLUS
DOCUMENT NUMBER: 121:250621
TITLE: Biospecific fluorescent **microparticle** immunoassay
INVENTOR(S): Iitiae, Antti Juhana; Loevgren, Timo Nils-Erik; Petterson, Kim Sverker Immanuel
PATENT ASSIGNEE(S): Wallac OY, Finland
SOURCE: Eur. Pat. Appl., 8 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 617286	A2	19940928	EP 1994-850024	19940216
EP 617286	A3	19950726		
EP 617286	B1	19991222		
R: DE, ES, FR, GB, IT, SE				
FI 9301198	A	19940919	FI 1993-1198	19930318
FI 93781	B	19950215		
FI 93781	C	19950526		
ES 2139728	T3	20000216	ES 1994-850024	19940216
JP 06317593	A2	19941115	JP 1994-47042	19940317
PRIORITY APPLN. INFO.:			FI 1993-1198	19930318

AB A biospecific multiparameter assay uses **microparticles** distributed in different categories and representing different **analytes**; the **microparticles** belonging to different categories are **labeled** with a 1st fluorescent mol. and are **coated** with bioaffinity reactants binding the different **analytes**. The **microparticles** and fluorescent-**labeled** bioaffinity reactants are **labeled** with different mols. emitting long-lived fluorescence. Thus, TSH, T3, T4, and thyroglobulin concns. were **detd**. simultaneously in blood **samples** with 4 **particle** categories of identical size and properties, **labeled** with different Tb chelate concns. and the following **reagents labeled** with a fluorescent Eu chelate: monoclonal antibodies specific for TSH and thyroglobulin, a T3

deriv., and a T4 analog. After incubation, the **particle** categories were identified on the basis of their Tb chelate concn., and the Eu concn. of individual identified **particles** was measured by time-resolved fluorescence using a **flow cytometer**, microscope, or microfluorometer. **Analyte** concns. were calcd. from the measured Eu concns.

L16 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:621158 CAPLUS
DOCUMENT NUMBER: 119:221158
TITLE: Formation of colloidal metal dispersions using aminodextrans as reductants and protective agents
INVENTOR(S): Siiman, Olavi; Burshteyn, Alexander
PATENT ASSIGNEE(S): Coulter Corp., USA
SOURCE: PCT Int. Appl., 44 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9315117	A1	19930805	WO 1993-US979	19930128
W: AU, BG, BR, CA, FI, HU, JP, KR, NO, PL, RO, RU				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5248772	A	19930928	US 1992-827347	19920129
IL 104455	A1	19960618	IL 1993-104455	19930120
ZA 9300525	A	19940725	ZA 1993-525	19930125
AU 9336089	A1	19930901	AU 1993-36089	19930128
EP 625993	A1	19941130	EP 1993-904879	19930128
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 07504843	T2	19950601	JP 1993-513509	19930128
CA 2128230	C	19990420	CA 1993-2128230	19930128
CN 1074844	A	19930804	CN 1993-100522	19930129

PRIORITY APPLN. INFO.: US 1992-827347 19920129
WO 1993-US979 19930128

OTHER SOURCE(S): MARPAT 119:221158

AB Colloidal metal(0) **particles** are prepd. by (a) heating a soln. contg. an amine-derivatized oxidizable sugar-contg. polysaccharide and a metal salt to reduce the metal salt to colloidal metal **particles** and simultaneously **coating** the **particles** with the polysaccharide; and (b) stabilizing the amine-derivatized polysaccharide **coating** on the **particles** by means of a bifunctional crosslinking

agent, a diamine, and a reducing agent. The **coated particles** can be used to covalently bind proteins for use as markers in optical and electron microscopy, in immunol. and biol. assays, etc. Dextran was partially cleaved and oxidized and then derivatized with 1,3-diaminopropane. A soln. of HAuCl4 and the aminodextran was boiled to make **coated** colloidal Au **particles** which were stabilized by treatment with glutaraldehyde, ethylenediamine, and Na borohydride. Monoclonal antibody was conjugated with the crosslinked and stabilized **coated particles**. The conjugates were used in anal. of T-cell subsets by polarized light microscopy and **flow cytometry**.

L16 ANSWER 16 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:463809 CAPLUS
DOCUMENT NUMBER: 119:63809
TITLE: GABAergic cells and signals appear together in the early post-mitotic period of telencephalic and striatal development
AUTHOR(S): Fiszman, Monica L.; Behar, Toby; Lange, G. David; Smith, Susan V.; Novotny, Elizabeth A.; Barker, Jeffery L.
CORPORATE SOURCE: Laboratory of Neurophysiology and, Bethesda, MD, 20892, USA
SOURCE: Dev. Brain Res. (1993), 73(2), 243-51
CODEN: DBRRDB; ISSN: 0165-3806
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Single cell suspensions derived from embryonic telencephala taken from embryos of gestational day 13 (E13) as well as rat striatal tissue from E14, 15 and 17 were prepd. by tissue digestion with papain. Cell suspensions were analyzed by **flow cytometry** or plated onto poly-D-lysine-**coated** culture dishes for either nuclear **staining** or immunocytochem. Expts. on functional Na+ channels and GABAA receptor expression were carried out using a fluorescence-activated cell sorter (FACS) and a neg. charged fluorescent indicator **dye** (oxonol). FACS anal. of embryonic cell suspensions at E13-17 consistently revealed one major subpopulation accounting for 85-90% of the events and one minor subpopulation (10-15% of the total). When **sorted**, the major **subpopulation** consisted of phase-bright cells of 5-7 .mu.m diam. some of which had neurites. The minor population consisted of phase-dark cells and resealed membranes of 0.5-4 .mu.m diam. as well as debris. Almost all the cells obtained in the high FALS (forward-angle light scatter) subpopulation at E17 expressed 200-kDa neurofilament and tetanus **toxin** antigens while the small diam. cells seldom expressed tetanus **toxin** and **particles** never did.

A small no. of GABA-contg. neurons were detected in the telencephalon at E13 (3%) and in the developing striatum at E14 (6%). All of the GABA-contg. neurons expressed neurofilament. In the embryonic rat striatum, nanomolar concns. of muscimol (GABAA agonist) induced depolarizing responses. A small no. of cells in the high FALS subpopulation were responsive to muscimol starting at embryonic day 14, and the no. of responsive cells increased at E15. At E17, both FALS subpopulations (high and low) were responsive to muscimol. A modest response to muscimol was obsd. in E13 telencephalic cells. A small percentage of GABA-contg. neurons was present in the cell suspension at this time. Fully developed GABAA-mediated responses at E15 coincided with an approx. 5-fold increase in the percentage of GABA-contg. neurons. Veratridine responses were already **detectable** in the high FALS **subpopulation** of E13 telencephalic cells.

L16 ANSWER 17 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1993:142986 CAPLUS
DOCUMENT NUMBER: 118:142986
TITLE: Methods and compositions for simultaneous analysis of multiple **analytes**, especially with **flow cytometry**
INVENTOR(S): Lehnen, Brian C.; Crothers, Stephan D.
PATENT ASSIGNEE(S): Transmed Biotech Inc., USA
SOURCE: PCT Int. Appl., 70 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9302360	A1	19930204	WO 1992-US5799	19920710
W: AU, CA, JP, NO, US				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE				
CA 2113350	AA	19930204	CA 1992-2113350	19920710
CA 2113350	C	19990323		
AU 9223480	A1	19930223	AU 1992-23480	19920710
EP 594763	A1	19940504	EP 1992-916006	19920710
EP 594763	B1	19980923		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
JP 06509417	T2	19941020	JP 1992-502870	19920710
AT 171543	E	19981015	AT 1992-916006	19920710
US 5567627	A	19961022	US 1993-149129	19931105
PRIORITY APPLN. INFO.:			US 1991-731039	19910716
			WO 1992-US5799	19920710

AB A method is disclosed for **detection** of multiple

analytes in a **sample** employing a complementary binding moiety to each of the **analytes** bound to a solid support, wherein each **analyte** and its complementary binding moiety comprise 1st and 2nd members of a specific binding pair. The method includes (1) forming a mixt. of known proportions of multiple subpopulations of the complementary binding moieties, in which each subpopulation comprises a different complementary binding moiety; (2) contacting the sample with the mixt. so that specific binding pairs are formed on the solid supports; and (3) relating the presence of **analytes** in the sample to the formation of specific binding pairs assocd. with each unique proportion of multiple subpopulations by comparing the area of the peak in the fluorescence histogram to the total area of peaks in the histogram. The method can be performed with solid supports of a single av. size and a single fluorochrome and without the need for using other detection systems. **Reagents** of **microspheres** **coated** with either human immunodeficiency virus (HIV) gp41 or with goat anti-human IgG were prepd. **Reagents** contg. exclusively either or both of these **coated** **microspheres** were blended proportionately so that gp41-**coated** **microspheres** comprised 100, 89, 79, 68, 58, 48,38,28,19,9, and 0% of the total no. of **microspheres** in the **reagent** and anti-IgG antibody-**coated** **microspheres** comprised 0, 11, 21, 32, 42, 52, 62, 72, 81, 91, and 100%, resp. Each of the 11 proportional **reagents** was incubated with human serum known to be pos. for both IgG and anti-gp41 antibodies; after washing, a 2nd **reagent**, contg. FITC-**labeled** anti-human IgG antibodies, was added and the the mixt. incubated, washed, and analyzed with a **flow** **cytometer**. The data were analyzed and output as a histogram; 1 or 2 histogram peaks was obsd., depending on whether the **reagent** contained 1 or 2 types of **microspheres** ,resp. Magnitudes of peak areas varied in a manner predicted by the proportionality of **microspheres** in the **reagents**. In other expts. it was shown that histogram area is independent of fluorescence intensity, that the summed area of overlapping peaks is detd. by the proportionality of the **reagent** **microspheres** whose fluorescence contributes to the combined peak in the histogram, and that the area of a histogram peak arising from nonspecific binding (i.e., a neg.) is detd. by the proportionality of the resp. **microspheres** in the **reagent**. A four-**analyte** serum assay using **microspheres** **coated** with IgG antibodies, HIV gp41, HIV p24, and hepatitis B core protein is also described, as are methods of data anal. for the method of the invention.

L16 ANSWER 18 OF 18 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1987:473848 CAPLUS

DOCUMENT NUMBER: 107:73848
TITLE: Quantitative analysis with **particle** agglutination tests
INVENTOR(S): Mizukoshi, Tatsuya; Ebisawa, Hisashi
PATENT ASSIGNEE(S): Showa Denko K. K., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 6 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 62081567	A2	19870415	JP 1985-221948	19851007

AB Simultaneous **detn.** of multiple substances in a **sample** by a **particle** agglutination test is based on the contact of the sample with a **reagent** contg. various **particles** sensitized with responsive substances that bind to corresponding test substances, and judgement of patterns of the agglutination produced by various-sized **particles** and(or) **particles labeled** with fluorescence substances or **dyes** by **flow cytometry**. Thus, a sample contg. human IgG and IgM was contacted with a **reagent** contg. anti-human IgG antibody-sensitized fluorescent substance-contg. polystyrene **beads** (2.98 .mu.m diam.) and anti-human IgM antibody-sensitized fluorescent substance-free polystyrene **beads** (2.95 .mu.m diam.), and the agglutination patterns of various **particles** were detd. by **flow cytometry** based on the fluorescent substance content for simultaneous detn. of IgG and IgM.

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO' ENTERED AT 11:40:33 ON 16 AUG 2001)
L17 2 S L3
L18 74 S L15
L19 75 S L17 OR L18
L20 59 DUP REM L19 (16 DUPLICATES REMOVED)

L20 ANSWER 1 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-367092 [38] WPIDS
DOC. NO. CPI: C2001-112474
TITLE: Screening, sequencing and/or quantitating a nucleic acid of interest by hybridizing the nucleic acid with a set of oligonucleotide probes bound to fluorescently addressable **microspheres** that are suspended in a fluid array.
DERWENT CLASS: B04 D16

INVENTOR(S): CHANDLER, M B
PATENT ASSIGNEE(S): (LUMI-N) LUMINEX CORP
COUNTRY COUNT: 90
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001014589	A2	20010301	(200138)*	EN	63

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000067881	A	20010319	(200139)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001014589	A2	WO 2000-US22769	20000821
AU 2000067881	A	AU 2000-67881	20000821

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000067881	A	Based on	WO 200114589

PRIORITY APPLN. INFO: US 1999-149710 19990820
AN 2001-367092 [38] WPIDS
AB WO 200114589 A UPAB: 20010711
NOVELTY - Screening, sequencing and/or quantitating a nucleic acid of interest by hybridizing the nucleic acid with a set of oligonucleotide probes bound to fluorescently addressable **microspheres** that are suspended in a fluid (e.g. liquid, suspension or gaseous array), is new. The identity of the probe is determined by the fluorescent signature of the **microparticle**

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a collection (C1) comprising subpopulations of **particles**, where the **particles** in each **subpopulation** have characteristics that **distinguish** the **particles** of the **subpopulations** and the collection is further characterized as having about 1000 or more distinct subpopulations of **particles**;

(2) a fluid array comprising:

(a) C1 which has bound nucleic acid; and
(b) a fluid carrier;
(3) a composition of matter comprising a solid **particle** including:
(a) bound nucleic acid having a known polynucleotide sequence;
(b) a **label** comprising a **dye** that exhibits a distinctive fluorescence signature; and
(c) a substance that, in the absence of an **analyte** of interest comprising a polynucleotide sequence complementary to the known polynucleotide sequence can quench the fluorescence emission of the **dye**;
(4) of characterizing (M1) a nucleic acid of interest, comprising:
(a) providing oligomer probes of known or ascertainable sequence, bound to a respective number of **subpopulations** of **particles** having characteristics that **distinguish** the **particles** of the **subpopulations** so that the sequence of a probe is identifiable according to the unique characteristic of the particular subpopulation of **particles**;
(b) hybridizing the oligomer probes with the nucleic acid of interest to obtain complementary complexes; and
(c) determining the sequence of the nucleic acid of interest in the complementary complexes by referring to the unique characteristic associated with each subpopulation of **particles** carrying the probe of known or ascertainable sequence;
(5) quantitating an **analyte** of interest in a sample comprising:
(a) contacting the **sample** with a **detectable** probe bound to a fluorescently addressable **particle**; and
(b) measuring the quantity of the **analyte** by comparing to a standard curve, where the standard curve comprises values from two known quantities of a reference **analyte**;
(6) an array of nucleic acid probes where each of the probes is bound to a discrete fluorescently addressable set of **microparticles**, each set is positioned in a predetermined well of a microtiter plate;
(7) a library of oligonucleotide probes of known sequence in which each discrete probe is bound to a respective fluorescent **microparticle stained** with fluorescent **dyes** and each **dye** has the potential of having eight different levels of fluorescence intensity;
(8) a device for identifying an **analyte** of interest among different **analytes** in a sample, comprising a fluorescently addressable **microparticle** having on its surface a bound probe of known sequence **labeled** with a fluorescent reporter **dye**, to which the **analyte**

of interest binds in complementary fashion so that the fluorescent reporter **dye** on the binding probe undergoes a change in fluorescence output indicating the presence of the **analyte** in the sample and the **analyte** is identified according to the fluorescent signature of the **microparticle**;

(9) constructing (M2) a library of oligomer probes of known sequence, comprising:

(a) coupling each of the four bases, A, C, G, and T, to four respective sets of fluorescently distinguishable **microparticles**;

(b) stacking by means of nucleotide synthesis chemistry to the **microparticle**-coupled base the next base selected from A, C, G or T;

(c) sorting **microparticles** according to the formed sequence; and

(d) repeating the nucleotide synthesis and sorting steps (b) and (c) until the desired sequence of the oligomer probe is obtained;

(10) constructing a library of oligomer probes of known sequence, comprising:

(a) synthesizing by nucleotide synthesis chemistry N number of sets of oligomer probes of desired sequence; and

(b) coupling an oligomer probe from one of the N number of sets of oligomer probes to a respective set of fluorescently distinct **microparticles labeled** with fluorescent **dyes** having eight different levels of fluorescence intensity;

(11) an array (A1) of nucleic acid probes comprising fluorescently addressable **microparticles**, each **stained** with fluorescent **dyes** and carrying a distinct nucleic acid probe, where the **microparticles** are arrayed in a two-dimensional pattern over a plane of a microtiter plate;

(12) a liquid array comprising a mixture of sets of fluorescently addressable microspheres in a liquid; and

(13) an enzymatic process for analyzing a nucleic acid sequence present in a sample of interest, comprising:

(a) providing an array of fluorescently addressable microparticles stained with distinct fluorescent dyes;

(b) hybridizing the nucleic acid in the sample of interest with the array; and

(c) analyzing the obtained hybrid by a primer extension enzymatic process.

USE - The method is useful for determining a genetic distance between the nucleic acid of interest and a reference sample. It is also useful for analyzing a nucleic acid of interest comprising at least one mutation or a set of mutations linked to a clinical condition or a predisposition to the clinical condition, where the

clinical is selected from hereditary diseases, neural diseases, muscle and bone diseases, malignant diseases, infectious diseases, metabolic diseases, or their combinations.

The arrays are useful in methods for carrying out sequencing by hybridization, for analyzing gene expression by hybridization of gene-specific mRNA or cDNA to an array of complementary probes, and for quantitating copies of nucleic acid sequences of interest by comparing to a known quantity of a reference material. The array is also useful for screening molecules that bind to array bound nucleic acids, where the molecules have various types of biological activities comprising hormonal, neurotransmitter, metabolic, genetic, pharmacologic, immunologic, pathologic, toxic, and anti-mitotic activities (all claimed).

ADVANTAGE - The methods do not suffer from the inherent limitations imposed by the two dimensional confinements of the gene chip technologies. The method also allows the resolution of up to 1000000 or more unique sets of particles, thus permitting the simultaneous detection of a corresponding number of probes bound to it.

The method is well suited to a multiplexing analysis format and is easily adapted to an automated procedure. The expenses and limitations associated with prior gene chip manufacturing and/or testing procedures are avoided.

Dwg.0/1

L20 ANSWER 2 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-168903 [17] WPIDS
DOC. NO. NON-CPI: N2001-121808
DOC. NO. CPI: C2001-050501
TITLE: **Microparticles** with multiple detectable **labels** for use in multiplex **assays** , to **detect** different **analytes** in **samples**.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): CHANDLER, D J; CHANDLER, M B
PATENT ASSIGNEE(S): (LUMI-N) LUMINEX CORP
COUNTRY COUNT: 90
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001013120	A1	20010222	(200117)*	EN	41

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000067797 A 20010313 (200134)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001013120 A1		WO 2000-US22543	20000817
AU 2000067797 A		AU 2000-67797	20000817

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000067797 A	Based on	WO 200113120

PRIORITY APPLN. INFO: US 1999-149225 19990817
AN 2001-168903 [17] WPIDS
AB WO 200113120 A UPAB: 20010328
NOVELTY - **Microparticles** with multiple detectable **labels** and methods of using them in multiplex assays (i.e. individual assays using differently **labeled microparticles** so they can be pooled and read out individually), are new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a carrier **particle** (I) comprising a **microparticle** and at least 1 **nanoparticle** of at least one **nanoparticle** population coupled with the **microparticle**;

(2) a population (II) of (I);

(3) a method (III) of determining concentration of an **analyte** in a sample, comprising:

(a) providing a population of carrier **particles** which include **microparticles** coupled with a subpopulation of **nanoparticles**, therefore providing a subpopulation of carrier **particles** (the subpopulation of **nanoparticles** is **labeled** to provide a specific **subpopulation** characteristic **detectable** by an instrument and the carrier **particles** of the specific carrier **particle** subpopulation has a **reagent** attached that reacts with the **analyte** to form a bimolecular product);

(b) contacting the sample with the population of carrier **particles**;

(c) identifying the subpopulation of the carrier **particles** using the specific subpopulation characteristic; and

(d) calculating the concentration of the **analyte** in

the **sample** according to the biomolecular product **detected** for the **subpopulation** of carrier **particles**;

(4) a kit (IV) for the detection of an **analyte** of interest, comprising at least 1 **microparticle** with attached **nanoparticles**;

(5) a method (V) of determining the concentration of an **analyte** in a number of samples of different origins using an instrument which separates and classifies **microparticles** and measures the results of bimolecular reactions between **analytes** and **reagents**, comprising:

(a) providing a population of **microparticles** which includes distinct subpopulations of **microparticles**, each subpopulation of which is uniquely **labeled** with 2 **labels** having 2 characteristic detectable by the instrument (the **microparticles** of each subpopulation have a **reagent** attached that reacts with one of the **analytes** with a bimolecular reaction, the results of the biomolecular reaction between **reagent** and **analyte** is measured in the instrument);

(b) exposing each sample to one subpopulation of **microparticles**;

(c) adding additional **reagents** to the mixture to facilitate a bimolecular reaction;

(d) incubating the mixture until the bimolecular reaction is complete;

(e) combining the reacted samples;

(f) passing the combined samples through the instrument;

(g) identifying the subpopulation of each **microparticle** using the 2 characteristics of each respective subpopulation;

(h) measuring the result of the **reagent** and **analyte** bimolecular reaction on each **microparticle** ; and

(i) calculating the concentration of **analyte** in each sample; and

(6) a method (VI) of determining the concentration of a number of **analytes** in a sample from a single origin using an instrument which separates and classifies **microparticles** and measures the results of a bimolecular reaction between an **analyte** and a **reagent**, comprising:

(a) providing a population of **microparticles** which includes distinct subpopulations of **microparticles**, each subpopulation of which is uniquely **labeled** with 2 **labels** having 2 characteristic detectable by the instrument (the **microparticles** of each subpopulation have a **reagent** attached that reacts with one of the **analytes** with a bimolecular reaction, the results of the biomolecular reaction between **reagent** and **analyte**

is measured in the instrument);
(b) mixing an aliquot of the sample with each subpopulation of **microparticles**;
(c) adding additional **reagents** to the mixture to facilitate a bimolecular reaction between an **analyte** and a **reagent**;
(d) incubating the mixture until the bimolecular reactions are complete;
(e) combining the reacted samples;
(f) passing the combined samples through the instrument;
(g) identifying the subpopulations of each **microparticle** using the characteristics of the subpopulation;
(h) determining the results of the reaction between **reagent** and **analyte** on each **microparticle** by measuring the result of the **reagent** and **analyte** bimolecular reaction and;
(i) calculating the concentration of the analyte in each sample.
USE - The microparticles are used in multiplex assays (i.e. individual assays using differently labeled microparticles so they can be pooled and read out individually) for the detection of analytes in samples.
ADVANTAGE - The microparticles are uniquely distinguished by detectable characteristics.
Dwg.0/0

L20 ANSWER 3 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2001-168815 [17] WPIDS
DOC. NO. NON-CPI: N2001-121731
DOC. NO. CPI: C2001-050469
TITLE: Identification of cellular response producing **compounds** involves using apparatus to form test mixtures, directing test mixtures through detection zone and measuring cellular responses to test **compound**.
DERWENT CLASS: B04 S03
INVENTOR(S): OKUN, A; OKUN, I; RANSOM, J
PATENT ASSIGNEE(S): (AXIO-N) AXIOM BIOTECHNOLOGIES INC
COUNTRY COUNT: 94
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001011333	A2	20010215	(200117)*	EN	141

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE

DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ
PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU
ZA ZW

AU 2000068937 A 20010305 (200130)
US 6242209 B1 20010605 (200133)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2001011333	A2		WO 2000-US21339	20000804
AU 2000068937	A		AU 2000-68937	20000804
US 6242209	B1	CIP of	US 1996-691356	19960802
		Cont of	US 1997-904904	19970801
		CIP of	US 1999-317793	19990524
		Cont of	US 1999-370786	19990805
			US 2000-568778	20000510

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 2000068937	A	Based on	WO 200111333
US 6242209	B1	CIP of	US 5804436
		Cont of	US 5919646
		CIP of	US 6096509

PRIORITY APPLN. INFO: US 1999-370786 19990805; US 1996-691356 19960802; US 1997-904904 19970801; US 1999-317793 19990524; US 2000-568778 20000510

AN 2001-168815 [17] WPIDS

AB WO 200111333 A UPAB: 20010328

NOVELTY - Cellular response producing **compounds** are identified by:

(1) using an apparatus for continuously contacting **particles**, to combine a suspension of cells with test **compound**(s) to form test mixtures;

(2) directing the test mixtures through a detection zone and

(3) measuring cellular responses to the test **compound**(s) in the suspended cells.

DETAILED DESCRIPTION - Identification of cellular response producing **compounds** comprises:

(1) using an apparatus for continuously contacting **particles**, to combine a suspension of cells with test **compound**(s) to form test mixtures;

(2) directing the test mixtures through a detection zone and

(3) simultaneously measuring cellular responses to the test

compound(s) in the suspended cells as the test mixtures are flowing through the detection zone.

The detection zone is capable of detecting cellular responses simultaneously.

An INDEPENDENT CLAIM is also included for an apparatus comprising a test **compound** source, a test substrate source, a mixing chamber in fluid communication with the test **compound** source and the test substrate source and a detector in communication with the mixing chamber. The mixing chamber combines a test **compound** received from the test **compound** source with a test substrate to generate a mixture. The detector is capable of detecting an interaction between the test **compound** and the test substrate while the mixture is passing through the detector.

USE - Used for identifying **compounds** which produce a cellular response e.g. activation or inhibition of receptor mediated response, activation or inhibition of an ion channel, activation or inhibition of non-selective pore, activation or inhibition of a second messenger pathway at a point downstream of a receptor or channel, activation or inhibition of apoptosis, or activation or inhibition of cellular necrosis, or cellular toxicity (claimed). The method can also be used for **determining** a **sample** containing molecules, e.g. polypeptides, nucleic acids, receptor ligands, enzymatic agonists, or enzymatic antagonists. It can also be used to perform biochemical analyses, e.g. western analyses, northern analyses, detection of single nucleotide polymorphins, detection of enzymatic activities, or molecular assembly assays.

ADVANTAGE - The method allows automated characterization of pharmacological profiles and corresponding potencies of **compounds** in synthesized combinatorial libraries. It also enables the rapid screening of a large number of **compounds** in the combinatorial library, and increases the number of samples which can be evaluated over a given period of time. The apparatus is capable of real-time variation of concentrations of test and standard **compounds** and generation of dose/response profiles within a short time span.

Dwg.0/33

L20 ANSWER 4 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 2001186857 EMBASE

TITLE: Generation of dopaminergic neurons in the adult brain from mesencephalic precursor cells **labeled** with a nestin-GFP transgene.

AUTHOR: Sawamoto K.; Nakao N.; Kakishita K.; Ogawa Y.; Toyama Y.; Yamamoto A.; Yamaguchi M.; Mori K.; Goldman S.A.; Itakura T.; Okano H.

CORPORATE SOURCE: Dr. H. Okano, Department of Physiology, Keio University School of Medicine, 35 Shinanomachi,

Shinjuku, Tokyo 160-8582, Japan.
hidokano@sc.itc.keio.ac.jp
SOURCE: Journal of Neuroscience, (1 Jun 2001) 21/11 (3895-3903).
Refs: 52
ISSN: 0270-6474 CODEN: JNRSDS
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 008 Neurology and Neurosurgery
021 Developmental Biology and Teratology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Mesencephalic precursor cells may one day provide dopaminergic neurons for the treatment of Parkinson's disease. However, the generation of dopaminergic neurons from mesencephalic precursors has been difficult to follow, partly because an appropriate means for recognizing mesencephalic ventricular zone precursors has not been available. To visualize and isolate mesencephalic precursor cells from a mixed population, we used transgenic mice and rats carrying green fluorescent protein (GFP) cDNA under the control of the nestin enhancer. Nestin-driven GFP was detected in the mesencephalic ventricular zone, and it colocalized with specific markers for neural precursor cells. In addition, data from **flow-cytometry** indicated that Prominin/CD133, a cell-surface marker for ventricular zone cells, was expressed specifically in these GFP-positive (GFP (+)) cells. After sorting by fluorescence-activated cell sorting, the GFP(+) cells proliferated in vitro and expressed precursor cell markers but not neuronal markers. Using clonogenic **sphere** formation **assays**, we showed that this **sorted population** was enriched in multipotent precursor cells that could differentiate into both neurons and glia. Importantly, many neurons generated from nestin-GFP-sorted mesencephalic precursors developed a dopaminergic phenotype in vitro. Finally, nestin-GFP(+) cells were transplanted into the striatum of a rat model of Parkinson's disease. Bromodeoxyuridine-tyrosine hydroxylase double-**labeling** revealed that the transplanted cells generated new dopaminergic neurons within the host striatum. The implanted cells were able to restore dopaminergic function in the host striatum, as assessed by a behavioral measure: recovery from amphetamine-induced rotation. Together, these findings indicate that precursor cells harvested from the embryonic ventral mesencephalon can generate dopaminergic neurons able to restore function to the chemically denervated adult striatum.

L20 ANSWER 5 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2001217707 EMBASE

TITLE: Social stress induces glucocorticoid resistance in macrophages.

AUTHOR: Stark J.L.; Avitsur R.; Padgett D.A.; Campbell K.A.; Beck F.M.; Sheridan J.F.

CORPORATE SOURCE: J.F. Sheridan, College of Dentistry, Section of Oral Biology, 305 W. 12th Ave., Columbus, OH 43218-2357, United States. sheridan.1@osu.edu

SOURCE: American Journal of Physiology - Regulatory Integrative and Comparative Physiology, (2001) 280/6 49-6 (R1799-R1805).
Refs: 31
ISSN: 0363-6119 CODEN: AJPRDO

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Stress-induced levels of plasma glucocorticoid hormones are known to modulate leukocyte function. These experiments examined the effects of a social stressor on the responsiveness of peripheral immune cells. Male mice experienced six evening cycles of social disruption (SDR), in which an aggressive male intruder was placed into their home cage for 2 h. Although circulating corticosterone was elevated in SDR mice, they had enlarged spleens and increased numbers of splenic leukocytes. Splenocytes from SDR and control mice were cultured with lipopolysaccharide and corticosterone. Cells from SDR mice exhibited decreased sensitivity to the antiproliferative effects of corticosterone, suggesting that the peripheral immune cells were resistant to glucocorticoids. In addition, SDR cells produced more interleukin (IL)-6. To **determine** which cell **population** was affected, we used antibody-**labeled** magnetic **beads** to deplete splenocyte suspensions of B cells or macrophages. Depletion of macrophages from SDR cultures, but not depletion of B cells, abolished both the corticosterone resistance and enhanced IL-6 secretion. These findings demonstrate that a psychosocial stressor induced glucocorticoid resistance in mouse splenic macrophages.

L20 ANSWER 6 OF 59 EMBASE

ACCESSION NUMBER: 2001238600 EMBASE

TITLE: **Flow cytometric microsphere**-based immunoassay: Analysis of secreted cytokines in whole-blood samples from asthmatics.

AUTHOR: Camilla C.; Mely L.; Magnan A.; Casano B.; Prato S.; Debono S.; Montero F.; Defoort J.-P.; Martin M.; Fert V.

CORPORATE SOURCE: C. Camilla, Beckman-Coulter Company, Immunoanalysis

Department, 130 av. de Lattre de Tassigny, 13276 Marseille, Cedex 9, France. camilla@immunotech.fr
SOURCE: Clinical and Diagnostic Laboratory Immunology, (2001) 8/4 (776-784).
Refs: 30
ISSN: 1071-412X CODEN: CDIMEN
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The ability of **flow cytometry** to resolve multiple parameters was used in a **microsphere**-based **flow cytometric** assay for the simultaneous **determination** of several cytokines in a **sample**. The **flow cytometer microsphere**-based **assay** (FMBA) for cytokines consists of **reagents** and dedicated software, specifically designed for the quantitative determination of cytokines. We have made several improvements in the multiplex assay: (i) dedicated software specific for the quantitative multiplex assay that processes data automatically, (ii) a stored master calibration curve with a two-point recalibration to adjust the stored curve periodically, and (iii) an internal standard to normalize the **detection** step in each **sample**. Overall analytical performance, including sensitivity, reproducibility, and dynamic range, was investigated for interleukin-4 (IL-4), IL-6, IL-10, IL-12, gamma interferon (IFN-.gamma.), and tumor necrosis factor alpha. These assays were found to be reproducible and accurate, with a sensitivity in the picograms-per-milliliter range. Results obtained with FMBA correlate well with commercial enzyme-linked immunosorbent assay data (r > 0.98) for all cytokines assayed. This multiplex assay was applied to the determination of cytokine profiles in whole blood from atopic and nonatopic patients. Our results show that atopic subjects' blood produces more IL-4 (P = 0.003) and less IFN-.gamma. (P = 0.04) than the blood of nonatopic subjects. However, atopic asthmatic subjects' blood produces significantly more IFN-.gamma. than that of atopic nonasthmatic subjects (P = 0.03). The results obtained indicate that the FMBA technology constitutes a powerful system for the quantitative, simultaneous determination of secreted cytokines in immune diseases.

L20 ANSWER 7 OF 59 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:214412 BIOSIS
DOCUMENT NUMBER: PREV200100214412
TITLE: Novel rapid method for visualization of extent and location of aerosol contamination during high-speed **sorting** of potentially biohazardous

samples.
AUTHOR(S): Oberyszyn, Andrew S. (1); Robertson, Fredika M.
CORPORATE SOURCE: (1) 410 West 12th Avenue, 416 Comprehensive Cancer Center, Columbus, OH, 43210: Oberyszyn.2@osu.edu USA
SOURCE: Cytometry, (March 1, 2001) Vol. 43, No. 3, pp. 217-222. print.
ISSN: 0196-4763.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Background: Containment of potentially biohazardous aerosols that result from high-speed sorting of human cells has been an increasingly important problem in analytical cytometry. The current method for assessing the efficiency of aerosol containment involves detection of aerosols containing sorted T4 bacteriophage on lawns of T4-susceptible Escherichia coli on plates that are placed in and around the sort area. Although this method is sensitive, it is time consuming and involves maintenance and handling of bacteria and sorting of bacteriophage that may themselves serve as sources of contamination for sorted viable human cells. Methods: Glo GermTM (5-mum melamine copolymer resin **beads**), which are fluorescent under black light illumination, were sorted on a Beckman-Coulter Elite ESP sorter in order to visualize deposition of aerosols under normal and mock failure modes. Results: Glo Germ was successfully used under both normal sorting conditions, as well as mock failure mode, to visualize aerosol formation. Conclusions: We have developed a method to examine aerosol containment using modified Glo Germ, a product used for teaching aseptic technique in hospitals, industry, restaurants, and schools. Use of this technique represents a rapid, inexpensive, qualitative analysis of the extent and location of aerosol contamination from cell sorters.

L20 ANSWER 8 OF 59 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 2001:281020 BIOSIS
DOCUMENT NUMBER: PREV200100281020
TITLE: Evaluation of autoimmune diseases using a multiple parameter latex **bead** suspension and **flow cytometry**.
AUTHOR(S): Hechinger, Mark K. (1)
CORPORATE SOURCE: (1) Pasadena, CA USA
ASSIGNEE: AffiniTech, LTD, Bentonville, AR, USA
PATENT INFORMATION: US 6159748 December 12, 2000
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 12, 2000) Vol. 1241, No. 2, pp. No Pagination. e-file.
ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English

AB Immunoassay methods and apparatus are provided which utilize **flow cytometry**, **coated** latex **microspheres**, and **labelled** antibodies, to simultaneously detect the presence and amount of several antigens or antibodies in a sample. **Microspheres** can be sized by forward angle light scatter (FALS) or electronic volume. By combining FALS and fluorescence, it is practical to use **beads** of several different sizes, colors or shapes, each **bead coated** with a different protein or antibody, for the simultaneous **detection** of multiple **analytes** in a **sample**. Available auto-sampling systems make it even more appealing in this regard. In accordance with one embodiment, highly purified RNP. Sm, SS-A, SS-B and Scl-70 antigens are bound to 4, 5, 6, 7 and 10 mum latex **beads**, respectively and stabilized for extended shelflife. Diluted patient serum is placed into test tubes containing a mixture of the five antigen **coated beads** and incubated. If an antibody is present for a specific antigen, it will bind to that specific **bead**. After washing the **bead**/serum mixture to remove residual sample, a second incubation with goat anti-human IgG, conjugated with a fluorochrone, is carried out. This conjugate will bind immunologically to the anti-antigen IgG of the antigen-antibody complex. forming a "sandwich" consisting of **bead**--antigen--primary antibody--secondary antibody--FITC. The fluorescence intensity is based on the avidity of the **bead**/antibody/conjugate binding. The samples are analyzed using a **flow cytometer**.

L20 ANSWER 9 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-258992 [23] WPIDS
CROSS REFERENCE: 2000-317371 [24]
DOC. NO. NON-CPI: N2000-192663
DOC. NO. CPI: C2000-079365
TITLE: Composition used for **detecting** biological **groups** e.g. target **analyte** comprises semiconductor nanocrystal core associated with first member of binding pair.
DERWENT CLASS: B04 D16 L03 S03
INVENTOR(S): BAWENDI, M G; MIKULEC, F V; SUNDAR, V C
PATENT ASSIGNEE(S): (MASI) MASSACHUSETTS INST TECHNOLOGY
COUNTRY COUNT: 25
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 990903	A1	20000405	(200023)*	EN	39

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

GB 2342651 A 20000419 (200023)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 990903	A1	EP 1999-307393	19990917
GB 2342651	A	GB 1999-22072	19990917

PRIORITY APPLN. INFO: US 1998-160454 19980924; US 1998-100947 19980918

AN 2000-258992 [23] WPIDS

CR 2000-317371 [24]

AB EP 990903 A UPAB: 20000725

NOVELTY - Composition (A) comprises semiconductor nanocrystal core associated with a first member of a binding pair.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) detecting target **analyte** which is a second member of the binding pair in a sample by admixing the **sample** with (A) and **detecting** binding of the first member of the binding pair and second member of the binding pair by monitoring the spectral emission of the sample, where the intensity and/or wavelength of the emission is related to the presence and/or amount of **analyte** in the sample;

(2) **labelling** a biological molecule or event with a fluorescent **label** comprising a semiconductor nanocrystal in which the emission spectrum of the fluorescence is dependent upon the nanocrystal size and

(3) controlling the fluorescence emission of a fluorescent group in use in a biological system which comprises selecting a semiconductor nanocrystal having a desired fluorescence emission spectrum and using the selected nanocrystal as the fluorescent group.

USE - The semiconductor nanocrystal is used as a fluorescent **label** in immunochemistry, optionally in immunocytochemistry or in an immunoassay, in DNA sequence analysis, in fluorescence resonance energy transfer in assessing the proximity of two or more biological **compounds** to each other, in **flow cytometry** or in a fluorescence activated cell sorter, in a diagnostic method or in biological imaging.

ADVANTAGE - A combination of tunability, narrow linewidths and symmetric emission spectra without a tailing region gives high resolution multiply sized nanocrystals and allows simultaneous examination of different biological groups e.g. target **analytes** tagged with nanocrystals. The range of excitation wavelengths of the nanocrystals is broad and can be higher in energy

than the emission wavelengths of all available semiconductor nanocrystals, which allows simultaneous excitation of all populations of nanocrystals in a system having distinct emission spectra with a single light source. The nanocrystals are more robust than organic fluorescent **dyes** and more resistant to photobleaching than organic **dyes**.
Dwg.0/10

L20 ANSWER 10 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000301206 EMBASE
TITLE: The clinical utility of fetal cell sorting to determine prenatally fetal E/e or e/e Rh genotype from peripheral maternal blood.
AUTHOR: Geifman-Holtzman O.; Makhlouf F.; Kaufman L.; Gonchorof N.J.; Holtzman E.J.
CORPORATE SOURCE: Dr. O. Geifman-Holtzman, Sheba Medical Center, Dept. of Obstetrics and Gynecology, Tel-Hashomer, Israel. Drgeifman@aol.com
SOURCE: American Journal of Obstetrics and Gynecology, (2000) 183/2 (462-468).
Refs: 22
ISSN: 0002-9378 CODEN: AJOGAH
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 010 Obstetrics and Gynecology
022 Human Genetics
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB OBJECTIVE: This study was undertaken to determine the fetal E/e or e/e Rh genotype prenatally from peripheral maternal blood by examining sorted fetal cells from alloimmunized and nonalloimmunized pregnancies. STUDY DESIGN: Eighteen maternal peripheral venous blood samples were obtained before amniocentesis from 15 pregnant women who were homozygous for the e allele. Five were not alloimmunized and 10 were alloimmunized. The mononuclear cell layer was isolated from the maternal blood and enriched for fetal nucleated red blood cells by **flow cytometry** with monoclonal antibodies to CD36 or CD71 and to glycophorin A. Eight samples were treated with CD45 monoclonal antibody-**coated** magnetic **beads** before they were **sorted** to deplete the maternal **sample** of leukocytes (CD45+ cells). We defined the positive fetal cell fractions as the monoclonal antibody positive-**sorted** cells derived from the maternal **samples**. These included **sorted** cells that were CD36+/glycophorin A+, CD71+/glycophorin A+ and CD45- cells that were sorted to become CD45-/CD36+/glycophorin A+ or CD45-/CD71+/glycophorin A+. The negative fractions were the cells that

were negative for either CD36/glycophorin A or CD71/glycophorin A or were the CD45+ cells. Deoxyribonucleic acid was isolated from all fractions and amplified by polymerase chain reaction with allele-specific primers for the E or e Rh genes. Gel electrophoresis was performed to detect fetal E/e or e/e Rh genotype. The fetal E/e or e/e Rh genotype was confirmed by serologic and deoxyribonucleic acid testing. The accuracy of E/e or e/e Rh genotype determination from the positive cell fractions was compared with that of E/e or e/e Rh genotype determination from the negative fractions. RESULTS: Fetal E/e or e/e Rh genotype was determined correctly in 17 of 18 of the fetal cell enriched positive fractions (94%). Fetal E/e or e/e Rh genotype was determined correctly in 11 of 14 of the maternal samples in the negative unselected cell fractions (79%). Fetal E/e or e/e Rh genotype was **determined** correctly in 15 of 16 **sample** fractions that underwent magnetic **bead separation** with CD45 and were subsequently sorted into positive and negative fractions (94%). Fetal E/e or e/e Rh genotype Was determined correctly in 13 of 13 of the samples obtained from the alloimmunized pregnancies (100%). CONCLUSIONS: The use of monoclonal antibodies for cell sorting or for magnetic separation predicted fetal E/e or e/e Rh genotype from peripheral maternal blood correctly in as many as 100% of alloimmunized pregnancies. Thus noninvasige fetal E/e or e/e Rh genotyping can be performed by polymerase chain reaction amplification of the rare fetal cells in maternal blood. The correct prediction of fetal E/e or e/e Rh genotype from the cell population not selected by the monoclonal antibodies suggests that there are fetal cell types other than fetal nucleated erythrocytes that can also be used as a source of fetal deoxyribonucleic acid for noninvasive genetic diagnosis. Improved technology may provide methods less laborious than cell sorting to accurately determine fetal Rh type from different fetal cell types that circulate in maternal blood.

L20 ANSWER 11 OF 59 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000411885 MEDLINE
DOCUMENT NUMBER: 20341020 PubMed ID: 10878568
TITLE: Characterization of **neurosphere** cell phenotypes by **flow cytometry**.
AUTHOR: Hulspas R; Quesenberry P J
CORPORATE SOURCE: Cancer Center and Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605, USA.. Ruud.Hulspas@umassmed.edu
CONTRACT NUMBER: CA68426-02 (NCI)
SOURCE: CYTOMETRY, (2000 Jul 1) 40 (3) 245-50.
Journal code: D92; 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000907
Last Updated on STN: 20000907
Entered Medline: 20000825

AB BACKGROUND: Neural stem cell research regularly utilizes **neurosphere** cultures as a continuous source of primitive neural cells. Results from current progenitor cell assays show that these cultures contain a low number of neural progenitors. Our goal is to characterize **neurosphere** cultures and define subpopulations in order to purify neural progenitor cells. METHODS: Cells from embryonic mouse **neurosphere** cultures were **stained** with Hoechst 33342 and analyzed by **flow cytometry**. **Subpopulations** were **sorted** based on their relative fluorescence intensity in the blue and red regions of the spectrum. Individual **sorted** **subpopulations** were reanalyzed after 7 days in culture. RESULTS: **Neurosphere** cultures contain a relatively high number of cells that **stain** weakly with Hoechst 33342. This subpopulation is present when cultured as an entire batch in the presence of epidermal growth factor (EGF). When cultured **separately**, this **subpopulation** gives rise to a **neurosphere** population with essentially the same characteristics as freshly isolated embryonic mouse brain cells but contains substantially fewer weakly Hoechst-**stained** cells. CONCLUSIONS: Similar to hemopoietic systems, **neurosphere** cultures contain a subpopulation that can be characterized by a low emission of Hoechst fluorescence. When cultured **separately** , this **subpopulation** gives rise to a phenotype similar to freshly isolated, uncultured neural cells.

L20 ANSWER 12 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000436816 EMBASE
TITLE: Preferential S phase entry and apoptosis of CD4+ T lymphocytes of HIV-1-infected patients after in vitro cultivation.
AUTHOR: Patki A.H.; Zielske S.P.; Sieg S.F.; Lederman M.M.
CORPORATE SOURCE: M.M. Lederman, Department of Medicine, Division of Infectious Diseases, University Hospitals of Cleveland, 2061 Cornell Road, Cleveland, OH 44106, United States. lederman.michael@clevelandactu.org
SOURCE: Clinical Immunology, (2000) 97/3 (241-247).
Refs: 29
ISSN: 1521-6616 CODEN: CLIIFY
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology

026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We have studied the relationship between spontaneous apoptosis and cell cycle perturbations in circulating peripheral blood lymphocytes of HIV-1-infected patients and healthy controls. PBMC obtained from HIV-1-infected patients and healthy controls were incubated in culture medium for 48 h. Cells were **separated** into CD4+ and CD8+ **populations** using immunomagnetic **beads**. Apoptosis and cell cycle phases were measured by propidium iodide **staining** and bromodeoxyuridine (BrdU) incorporation followed by **flow cytometric** analyses. In experiments using cells obtained from HIV-1-infected patients, spontaneous apoptosis was more frequent in CD4+ T lymphocytes than in CD8+ T lymphocytes (17.6% vs 9.5%, P < 0.005). Among healthy controls, spontaneous apoptosis in CD4+ and CD8+ T lymphocytes was comparable (4.5% vs 5.1%). Lymphocytes obtained from patients were more frequently in S phase than healthy controls' cells (2.2 .+-. 0.9% vs 0.5 .+-. 0.2%, P < 0.002) and patients' CD4+ cells tended to enter S phase more frequently than controls' CD4+ cells (4.2% .+-. 3.5% vs 1.8% .+-. 0.5% P < 0.04), whereas the frequency of S phase CD8+ T cells was not different among patients (2.8% .+-. 2.9%) and controls (1.8% .+-. 0.5%) (P > 0.4). Kinetic analyses using BrdU and PI **staining** revealed that S phase cells were more likely to become apoptotic than resting (G0-G1) cells (28.4% .+-. 10.3% vs 11.3% .+-. 9.9% in patients, P < 0.04, and 15.3% .+-. 2.8% vs 1.8% .+-. 0.5% in controls, P < 0.003). Lymphocytes obtained from HIV-1-infected persons are activated in vivo to enter S phase and to undergo spontaneous apoptosis after brief in vitro cultivation. The present studies indicate that most apoptotic cells in this system are CD4+ and kinetic analyses reveal that S phase cells are more likely to undergo spontaneous apoptosis than G0-G1 cells. Accelerated cell death in HIV-1 disease may contribute to the failure of lymphocyte responsiveness to appropriate T cell receptor stimulation. (C) 2000 Academic Press.

L20 ANSWER 13 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000389114 EMBASE
TITLE: A novel two-color **flow cytometric** assay for the **detection** of Cryptosporidium in environmental water **samples**.
AUTHOR: Ferrari B.C.; Vesey G.; Davis K.A.; Gauci M.; Veal D.
CORPORATE SOURCE: B.C. Ferrari, Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia. Bferrari@rna.bio.mq.edu.au
SOURCE: Cytometry, (1 Nov 2000) 41/3 (216-222).
Refs: 22
ISSN: 0196-4763 CODEN: CYTODQ

COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
027 Biophysics, Bioengineering and Medical Instrumentation
046 Environmental Health and Pollution Control
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Background: Cryptosporidium is an important waterborne pathogen, **Detection** of Cryptosporidium in concentrated water **samples** depends on oocyst isolation using immunomagnetic separation (IMS) and/or fluorescence-activated cell sorting (FACS), followed by confirmation using immunofluorescence **staining** (IFA) and fluorescence microscopy. These methods require highly trained microscopists for oocyst identification and confirmation. Analysis is hampered due to the presence of autofluorescent **particles** coupled with **particles** binding nonspecifically with the monoclonal antibodies (mAbs) used for detection. **Flow cytometry** (FCM) has the potential to be a more specific method for oocyst detection, but such a system would require more than one selection parameter. Methods: Various mAbs from commercial suppliers were paired with CRY104-PE and evaluated. The mAb combination that best discriminated **stained** oocyst from detritus was optimized and compared to Cryptosporidium detection utilizing one-color IFA/FACS. Results: A highly specific two-color assay employing the IgG1 mAb CRY104 was developed. The assay resulted in reductions, up to 20-fold, in the number of non-Cryptosporidium **particles** detected. The addition of a second selection parameter improved microscopic analysis times and simplified oocyst confirmation by microscopists. Conclusions: A two-color assay employing competing surface mAbs reduces the number of fluorescent **particles** sorted, thus improving FCM detection methods for Cryptosporidium. (C) 2000 Wiley-Liss, Inc.

L20 ANSWER 14 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 2000248311 EMBASE
TITLE: Multisite comparison of methods for the quantitation of the surface expression of CD38 on CD8+ T lymphocytes.
AUTHOR: Schmitz J.L.; Czerniewski M.A.; Ediriger M.; Plaeger S.; Gelman R.; Wilkening C.L.; Zawadzki J.A.; Wormsley S.B.; Asthana D.; Bauer Y.; Blair P.; Blais B.; Brennan T.; Bucy P.; Campbell.D.; Chiu S.; Czerniewski M.; Denny T.; Franks P.; Gonzaga C.; Helm K.; Kagan J.; Landay A.; Larson E.; Livnat D.; Luzuriaga K.; McCloskey T.; McFarland B.; Nichols J.; Nickishcer D.; Nokta M.; Pahwa S.; Patki A.; Perfetto

S.; Quinn T.; Reimann K.; Roberts N.; Rosenblatt H.; Sabath D.; Schmitz J.; Sevin A.; Spina C.; Thomas J.; Valentine F.; Weng D.; Zhang B.

CORPORATE SOURCE: J.L. Schmitz, Univ. of North Carolina Hospitals, 101 Manning Drive, Chapel Hill, NC 27514, United States. jschmitz@unch.unc.edu

SOURCE: Communications in Clinical Cytometry, (15 Jun 2000) 42/3 (174-179).
Refs: 17
ISSN: 0196-4763 CODEN: CCCYEM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We evaluated the effect of specimen processing variations and quantitation methods on quantitative determination of CD38 expression on CD8 T lymphocytes. Neither lysing **reagent** (ammonium chloride versus BD FACSlyse), fixation (paraformaldehyde versus no final fixation step), nor acquisition delay (acquisition within 6 h after fixation versus 24 h after fixation) had a significant effect on CD38 relative fluorescent intensity or CD38 quantitative estimates (RFI or antibodies bound per cell). The only significant difference in fluorescent intensity and CD38 antibodies bound per cell (ABC) was encountered when whole blood was held for 24 h prior to **staining** and fixation and then acquired after another 24-h hold. However, for all sample processing methods above, the CD4 biologic calibrator and QuantiBRITE **bead** methods gave significantly different estimates of CD38 intensity. In many cases, however, these differences are relatively small and were more pronounced in certain laboratories. We conclude that there is some flexibility in **sample** processing methods for quantitative CD38 **determination**; however, it is preferable for a laboratory to employ one method of fluorescence quantitation calculation consistently because small differences are detected between different methods. (C) 2000 Wiley-Liss, Inc.

L20 ANSWER 15 OF 59 MEDLINE DUPLICATE 2

ACCESSION NUMBER: 2000427146 MEDLINE
DOCUMENT NUMBER: 20394803 PubMed ID: 10938890

TITLE: Blood platelet activation evaluated by **flow cytometry**: optimised methods for clinical studies.

AUTHOR: Hagberg I A; Lyberg T
CORPORATE SOURCE: Ullevaal University Hospital, Oslo, Norway.. ingeranne.hagberg@ulleval.no

SOURCE: PLATELETS, (2000 May) 11 (3) 137-50.
Journal code: DSJ; 9208117. ISSN: 0953-7104.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000922
Last Updated on STN: 20000922
Entered Medline: 20000914

AB A variety of **flow cytometry** techniques are in use to evaluate in vivo blood platelet activation. We have in this study further developed and optimised these methods to be suitable for use in clinical studies. By preloading the Monovette EDTA vacuum blood sampling tubes with 1/8 vol 4% (w/v) paraformaldehyde (PFA), we were able to assess platelet CD62P (P-selectin) expression in whole blood with less than 0.2% activated platelets. No washing or neutralising steps were required to remove excess fixative. Both basal and agonist-stimulated CD62P expression were stable for at least 48 h after sampling. The standard curve was linear from 1.9 (basal) to 8.1 x 10(3) (TRAP-stimulated) molecules of equivalent soluble fluorochrome units (MESF) in phycoerythrein-conjugated anti-CD62P **labelled** whole blood **samples**. These **assay** conditions were also well suited for assessment of platelet expression of CD41, CD42a, CD61 and CD63. The preanalytic storage period was extended from 10 min to at least 2 h for platelet PAC-1 and fibrinogen binding analysis by preloading Monovette citrate tubes with 8/10 vol buffer. With PFA preloading, blood sampled into citrated tubes could be analysed for fractions of **microparticles** and platelet-platelet aggregates as well as for aggregate size.

L20 ANSWER 16 OF 59 MEDLINE
ACCESSION NUMBER: 2000395605 MEDLINE
DOCUMENT NUMBER: 20273613 PubMed ID: 10816101
TITLE: Two-photon fluorescence excitation in detection of biomolecules.
AUTHOR: Soini E; Meltola N J; Soini A E; Soukka J; Soini J T; Hanninen P E
CORPORATE SOURCE: Department of Medical Physics and Chemistry, University of Turku, Finland.
SOURCE: BIOCHEMICAL SOCIETY TRANSACTIONS, (2000 Feb) 28 (2) 70-4.
Journal code: E48; 7506897. ISSN: 0300-5127.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824

Last Updated on STN: 20000824
Entered Medline: 20000811

AB Two-photon fluorescence excitation has been found to be a very powerful method for enhancing the sensitivity and resolution in far-field light microscopy. Two-photon fluorescence excitation also provides a substantially background-free detection on the single-molecule level. It allows direct monitoring of formation of **labelled** biomolecule complexes in solution. Two-photon excitation is created when, by focusing an intensive light source, the density of photons per unit volume and per unit time becomes high enough for two photons to be absorbed into the same chromophore. In this case, the absorbed energy is the sum of the energies of the two photons. In two-photon excitation, **dye** molecules are excited only when both photons are absorbed simultaneously. The probability of absorption of two photons is equal to the product of probability distributions of absorption of the single photons. The emission of two photons is thus a quadratic process with respect to illumination intensity. Thus in two-photon excitation, only the fluorescence that is formed in the clearly restricted three-dimensional vicinity of the focal point is excited. We have developed an assay concept that is able to distinguish optically between the signal emitted from a **microparticle** in the focal point of the laser beam, and the signal emitted from the surrounding free **labelled reagent**. Moreover, the free **labels** outside the focal volume do not contribute any significant signal. This means that the assay is separation-free. The method based on two-photon fluorescence excitation makes possible fast single-step and separation-free immunoassays, for example, for whole blood **samples**. Since the method allows a **separation**-free assay in very small volumes, the method is very useful for high-throughput screening assays. Consequently we believe that two-photon fluorescence excitation will make a remarkable impact as a research tool and a routine method in many fields of analysis.

L20 ANSWER 17 OF 59 MEDLINE
ACCESSION NUMBER: 2000343178 MEDLINE
DOCUMENT NUMBER: 20343178 PubMed ID: 10884799
TITLE: A rapid and accurate closed-tube immunoassay for platelets on an automated hematology analyzer.
AUTHOR: Gill J E; Davis K A; Cowart W J; Nepacena F U; Kim Y R
CORPORATE SOURCE: Research & Development Department, Abbott Diagnostics Division, Santa Clara, CA 95054, USA.
SOURCE: AMERICAN JOURNAL OF CLINICAL PATHOLOGY, (2000 Jul) 114 (1) 47-56.
Journal code: 3FK; 0370470. ISSN: 0002-9173.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200007
ENTRY DATE: Entered STN: 20000728
Last Updated on STN: 20000728
Entered Medline: 20000719

AB Accurate and precise platelet counts are important for patients with severe thrombocytopenia or who are receiving chemotherapy. We developed a novel **flow cytometric** analysis of platelets that may be particularly valuable for assessing the necessity for platelet transfusions. This ImmunoPlt (CD61) assay is based in part on CD61 monoclonal antibody **labeling** and has been automated and implemented on the CELL-DYN 4000 hematology analyzer. It is well suited for thrombocytopenic specimens, since it reduces interference by nonplatelet **particles**. It takes less than 5 minutes from closed-tube aspiration to report. Data for more than 350 thrombocytopenic **specimens** demonstrate that the ImmunoPlt (CD61) **assay** is more accurate than the optical scatter or the impedance count for specimens with platelet counts between 1 and 60 x 10(3)/microL (1 and 60 x 10(9)/L). The ImmunoPlt (CD61) assay is more precise than the optical scatter or the impedance count for specimens with platelet counts between 1 and 50 x 10(3)/microL (1 and 50 x 10(9)/L).

L20 ANSWER 18 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-126491 [11] WPIDS
DOC. NO. NON-CPI: N2000-095342
DOC. NO. CPI: C2000-038501
TITLE: New multiple assay method for screening **drugs**.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): THOMAS, N
PATENT ASSIGNEE(S): (AMSH) AMERSHAM PHARMACIA BIOTECH UK LTD
COUNTRY COUNT: 22
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9964867	A1	19991216	(200011)*	EN	26
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA JP US					
EP 1036332	A1	20000920	(200047)	EN	
R: CH DE ES FR GB IT LI NL SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE

WO 9964867	A1	WO 1998-GB3727	19981203
EP 1036332	A1	EP 1998-959060	19981203
		WO 1998-GB3727	19981203

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 1036332	A1 Based on	WO 9964867

PRIORITY APPLN. INFO: EP 1997-309784 19971204
AN 2000-126491 [11] WPIDS
AB WO 9964867 A UPAB: 20000807
NOVELTY - **N samples** where N at least 2, each containing a **compound** to be tested are dispensed in different reaction vessels, provided with N number of distinguishable carrier **beads** and analyzed by **flow cytometry**.

DETAILED DESCRIPTION - The **assay** method comprises dispensing **N populations** of **distinguishable** carrier **beads** into one of N different reaction vessels, dispensing each of the **N samples** into the different reaction vessels, providing **reagents** where the signal moiety is partitioned in a **compound**-related manner between the carrier **beads** in the reaction vessel and a supernatant fluid, combining the contents of all of the reaction vessels into a mixture and subjecting the mixture to **flow cytometric** analysis, to assay the signal moiety associated with each of a sequence of individual **beads**.

An INDEPENDENT CLAIM is also included for the kit for the **assay** of **N samples** comprising **assay reagents** and **N populations** of **distinguishable** carrier **beads**, **precoated** with the **sample reagent** for performing the **assay**.

USE - The multiassay method is useful for performing high-throughput screening of **drugs** and for determining the concentration, biological activity of **compounds**.

ADVANTAGE - By using a number of discrete **bead** types, standard **flow cytometry** can be used to identify the **bead** type and to measure the assay signal associated with each **bead**, hence facilitating several tests in parallel on a single sample. Each **bead** in any individual well is identical to every other **bead** in the same well and thereby serves as an individual assay unit separately measurable by **flow cytometry**.

Dwg.1/3

L20 ANSWER 19 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-072353 [06] WPIDS
CROSS REFERENCE: 1999-081405 [07]; 2000-053005 [02]
DOC. NO. NON-CPI: N2000-056629
DOC. NO. CPI: C2000-020625
TITLE: Multi-**analyte** diagnostic system for use with a computer.
DERWENT CLASS: B04 D16 J04 S03 S05 T01
INVENTOR(S): CHANDLER, V S
PATENT ASSIGNEE(S): (LUMI-N) LUMINEX CORP
COUNTRY COUNT: 82
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9958955	A1	19991118	(200006)*	EN	168

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SL SZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG UZ VN YU ZA ZW

AU 9938979	A	19991129	(200018)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9958955	A1	WO 1999-US10316	19990513
AU 9938979	A	AU 1999-38979	19990513

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9938979	A	Based on	WO 9958955

PRIORITY APPLN. INFO: US 1998-85381 19980514
AN 2000-072353 [06] WPIDS
CR 1999-081405 [07]; 2000-053005 [02]
AB WO 9958955 A UPAB: 20000412
NOVELTY - Multi-**analyte** diagnostic system comprises a flow analyzer (25) having a co-planar optical assembly with at least one light source and one optical detector; and a memory medium readable by the computer (900) that stores computer instructions including simultaneously processing biological **sample** and

determining the presence and quantity of at least one **analyte** of interest in the sample.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for (A) a cuvette holder; (B) a computer program product for use with a flow analyzer and a computer, which comprises a memory medium, a computer program containing instructions for (i) processing a biological sample through a flow analyzer, (ii) collecting data related to at least one characteristic classification parameter including subset data on fluorescence emission intensities, (iii) collecting data related to a presence or absence of an **analyte** of interest, and (iv) identifying and quantifying at least one **analyte** of interest, an application programming interface library, and a mathematics library; (C) a multi-**analyte** analysis or diagnostic method comprising (i), (ii), (iii), and (iv) as in (A), and reducing the spectral overlap to identify each **bead** according to its subset; (D) a **flow cytometer** comprising a base section, light sources, selectors, and a sample viewing chamber; (E) an analysis or diagnostic system comprising an initialization system which includes a termination system, a **bead** map file system, a reset system, and a user **bead** component system that acquires **bead** statistics; a machine control and monitoring system comprising a panel setting system that maintains current **flow cytometer** settings in a buffer or storage area, and a change panel setting system; and a sample acquisition and reporting system that includes a test start system that indicates when to begin collecting data from the machine control and monitoring system, a test stop system indicating when to stop collecting data, a test storage system that stores data in another buffer or storage area, and a test query system that performs the analysis or diagnosis on the data responsive to a predetermined program or user query; (F) an analysis or diagnostic method; (G) a detector apparatus comprising a U-block assembly, at least one optical beam splitter, at least one optical detector, and at least one push-pull assembly; and (H) a de-bubbler, in a flow analyzer having a pressure sensor and a sheath fluid reservoir, which comprises a bottle and a waterproof vent.

USE - The invention is useful with computers.

ADVANTAGE - The light source setup, e.g. the laser setup, is very stable, thus a fairly accurate spot on the fluid flow stream for reading the **beads** is obtained, and low power lasers can be used. A compressor with fairly low psi rating, less expensive, more compact, and longer lasting is used relative to other cytometers that require very expensive compressors with high psi ratings. The optical assembly which comprises **stainless** steel, is made of many different pieces, but are optionally bolted together onto one solid piece that holds the cuvette, the viewing chamber, the lasers, and the detectors, all securely mounted

together. The laser/detector assembly is compact, durable, and is easily shipped with little or no functional damage. The invention eliminates the variability of human judgment and subjectivity from the data collection and analysis by simultaneously performing data collection, **bead** set classification, and analysis techniques. It uses a flow analyzer which is a fraction of the size, weight, and cost of conventional **flow cytometers** . It provides a system that is a multiple times faster as conventional **flow cytometers**, and that reduces dead time to zero.

DESCRIPTION OF DRAWING(S) - The figure is a general schematic of the novel diagnostic system.

Flow analyzer 25

Computer 900

Dwg.4/50

L20 ANSWER 20 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1999-494283 [41] WPIDS

CROSS REFERENCE: 1998-130827 [12]; 2000-146398 [08]

DOC. NO. NON-CPI: N1999-368180

DOC. NO. CPI: C1999-144887

TITLE: **Separating** cells or cleaning nucleic acid **samples** in integrated electrophoretic microdevice having selective capture region, e.g. for isolating hematopoietic cells from bone marrow.

DERWENT CLASS: B04 D16 S03

INVENTOR(S): HAUSER, A K; HOOPER, H H; NELSON, R J; SASSI, A P; SINGH, S; WILLIAMS, S J

PATENT ASSIGNEE(S): (ACLA-N) ACLARA BIOSCIENCES INC

COUNTRY COUNT: 23

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9940174	A1	19990812	(199941)*	EN	50
RW: AT BE CH CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP					
AU 9924887	A	19990823	(200005)		
US 6074827	A	20000613	(200035)		
EP 1053298	A1	20001122	(200061)	EN	
R: BE CH DE ES FR GB IT LI NL					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9940174	A1	WO 1999-US2099	19990201
AU 9924887	A	AU 1999-24887	19990201

US 6074827	A	CIP of	US 1996-690307	19960730
		CIP of	US 1997-902855	19970730
			US 1998-18918	19980205
EP 1053298	A1		EP 1999-904501	19990201
			WO 1999-US2099	19990201

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9924887	A	Based on	WO 9940174
US 6074827	A	CIP of	US 5770029
EP 1053298	A1	Based on	WO 9940174

PRIORITY APPLN. INFO: US 1998-18918 19980205; US 1996-690307 19960730; US 1997-902855 19970730

AN 1999-494283 [41] WPIDS

CR 1998-130827 [12]; 2000-146398 [08]

AB WO 9940174 A UPAB: 20001128

NOVELTY - Separating cell types or cleaning up nucleic acid samples using an integrated electrophoretic microdevice having at least one enrichment region and a flowpath that directs waste flow away from the main electrophoretic flowpath.

DETAILED DESCRIPTION - A micromixture of two cell types (A, B) is analyzed using substrate having at least one microchannel which is branched to define two parallel paths, each with an enrichment region (ER) and a downstream detection region (DR). The mixture of cells is transferred electrokinetically to the two paths so that one cell type is retained in one ER and the other in the second ER. The untrapped cells are transported electrokinetically from ER and DR, then the retained cells transported similarly to DR, so that both cell types are simultaneously separated and analyzed.

An INDEPENDENT CLAIM is also included for clean-up of a nucleic acid (I) sample by applying it to a microchannel, in a substrate, that has:

(a) an ER, where (I) is contacted with many affinity binding capture and release agents, so that some (I) is separated from waste components, and

(b) a working region (WR) through which the waste portion of the sample does not flow and to which the captured portion of (I) is transported for processing and analysis.

USE - The method is useful for high-throughput screening for genetic diseases; **drug** discovery/development; in vitro diagnosis (e.g. **flow cytometery** of leucocyte subpopulations in subjects infected with human immune deficiency virus); molecular genetics analysis; nucleic acid diagnostics; cell separation (including isolation and purging, e.g. of hematopoietic cells from bone marrow or blood) and generally for biological

research.

ADVANTAGE - The device can provide concentration or clean-up of a sample, and serve as a reactor for target **analytes**. Unlike conventional affinity separation, the capture and separation steps are separate, i.e. two distinct specificities are required for each separation, making it possible to perform separations which were previously difficult or impossible. Contamination by non-selected cells or **compounds** is avoided and many analyses may be performed on-line.

DESCRIPTION OF DRAWING(S) - Schematic diagram of typical separation device.

Secondary flowpath (60,61) Electrodes controlling movement along secondary flowpath(62) Enrichment zone for **analyte** (57) Reservoir for eluent (52) Main flowpath (58,59) Electrodes for moving **analyte** along main flowpath (65) Detection zone 55 Dwg.4/27

L20 ANSWER 21 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-479080 [40] WPIDS
DOC. NO. NON-CPI: N1999-356676
DOC. NO. CPI: C1999-140968
TITLE: Detecting **analyte** using liposomes that contain signal generators activated by interaction by **analyte** and activator, e.g. for detecting explosives and microorganisms.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): AOJULA, H S; CLARKE, D J; LLOYD, C J; NICKLIN, S; TSILOSANI, M; WILSON, M T
PATENT ASSIGNEE(S): (MINA) UK SEC FOR DEFENCE; (MINA) UK SEC FOR DEFENCE EVALUATION & RES AGEN
COUNTRY COUNT: 86
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9938009	A1	19990729	(199940)*	EN	110
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
ZA 9900325	A	19990929	(199947)		70
AU 9921770	A	19990809	(200001)		
NO 2000003709	A	20000921	(200056)		
EP 1049932	A1	20001108	(200062)	EN	
R: AT BE CH DE DK ES FI FR GB IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9938009	A1	WO 1999-GB208	19990121
ZA 9900325	A	ZA 1999-325	19990118
AU 9921770	A	AU 1999-21770	19990121
NO 2000003709	A	WO 1999-GB208	19990121
		NO 2000-3709	20000719
EP 1049932	A1	EP 1999-901770	19990121
		WO 1999-GB208	19990121

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9921770	A	Based on	WO 9938009
EP 1049932	A1	Based on	WO 9938009

PRIORITY APPLN. INFO: GB 1998-1120 19980121

AN 1999-479080 [40] WPIDS

AB WO 9938009 A UPAB: 19991122

NOVELTY - An **analyte** (I) is **detected** by contacting a test **sample** with a containment system (CS) having a barrier that separates signal-generating **reagent** (SGR) from the sample, in presence of an element (E) that interacts specifically with (I). Interaction of (I) and E activates SGR, inside CS, and any signal generated (and retained) within CS is **detected** from the **sample** side of the barrier.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) CS for use in this process;

(2) kit for the process containing CS and E;

(3) method for generating a measurable signal by interaction of cobalt ions with a **dye** and measuring fluorescent signal caused by the interaction;

(4) method for modifying a signal from an SGR that generates a signal following reaction with a chemical activator by incubating SGR, before activation, with an ion or surfactant and/or in buffer at a pH that modifies a property of the signal; and

(5) detecting a chemical that modifies the signal from SGR by incubating SGR with test sample and measuring the resulting signal.

USE - The method is particularly used to detect (I) present in very low concentrations, e.g. chemical or biological agents, such as explosives, microorganisms (or their **toxins**), chemical warfare agents, pesticides, hormones and **drugs**.

ADVANTAGE - Containment in CS provides concentration of the signal and thus easier detection at increased sensitivity, so that

one or very few (I) will produce a measurable signal.
Dwg.0/44

L20 ANSWER 22 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-527217 [44] WPIDS
DOC. NO. NON-CPI: N1999-390527
DOC. NO. CPI: C1999-154815
TITLE: New carrier **microparticles** having attached **nanoparticles labeled** with a fluorescent **dye** for detection of **analytes**.
DERWENT CLASS: B04 D12 D13 D15 D16 J04 S03
INVENTOR(S): CHANDLER, D; CHANDLER, M B; CHANDLER, D J
PATENT ASSIGNEE(S): (LUMI-N) LUMINEX CORP
COUNTRY COUNT: 82
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9937814	A1	19990729	(199944)*	EN	40
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE GH GM HR HU ID IL IN IS JP KE KG KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW					
AU 9924642	A	19990809	(200001)		
EP 1049807	A1	20001108	(200062)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
US 6268222	B1	20010731	(200146)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9937814	A1		WO 1999-US1315	19990122
AU 9924642	A		AU 1999-24642	19990122
EP 1049807	A1		EP 1999-904191	19990122
			WO 1999-US1315	19990122
US 6268222	B1	Provisional	US 1998-72160	19980122
			US 1999-234841	19990122

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9924642	A	Based on	WO 9937814
EP 1049807	A1	Based on	WO 9937814

PRIORITY APPLN. INFO: US 1998-72160 19980122; US 1999-234841 19990122
AN 1999-527217 [44] WPIDS
AB WO 9937814 A UPAB: 19991026
NOVELTY - A novel article comprises a carrier **microparticle** having attached to it a predetermined amount of **nanoparticles labeled** with at least one fluorescent **dye**.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method of making a fluorescent article comprising attaching to a carrier polymer **microparticle** at least one set of polymer **nanoparticles**, each set of the **nanoparticles** having a distinct fluorescent signal;

(2) a method for determining the presence or absence of at least one **analyte** in a sample, comprising:

(a) mixing the sample and known amounts of **microparticles** having disposed on it at least one fluorescently **labeled nanoparticle** and at least one analytical reactant which binds or reacts with a respective **analyte**, to form a reaction mixture; and

(b) analyzing **microparticles** having reacted or bound the **analyte**, to establish the presence or absence of the **analyte** in the sample;

(3) a method of **detecting analytes** in a **sample**, each of the **analytes** being recognized by a respective analytical reactant, comprising:

(a) contacting the sample with populations of fluorescent articles, each population of the articles having a distinct fluorescent signal and a distinct analytical reactant, where, the analytical reactant specifically binds one of the **analytes** in the sample, each fluorescent article comprising at least one **nanoparticle labeled** with a respective fluorescent **dye**;

(b) adding the sample to a **label reagent**;

(c) analyzing the articles to detect the **label**, which indicates the binding of the **analyte** to the analytical reactant, and

(d) simultaneously **determining** the **populations** of articles having bound the respective **analyte** as a function of the distinct signal associated with each population; and

(4) a kit suitable for use in detection of **analytes** of interest, the kit comprising:

(a) a series of **microparticles** with attached **nanoparticles** having a distinct fluorescent signal and each member in the series having an analytical reactant capable of

specifically binding with one of **analytes** of interest;

(b) a secondary **reagent** comprising a **reagent** which binds to the same **analyte** as the analytical **reagent**; and

(c) a fluorescent **label** provided on or by, or associated with, the secondary **reagent** where, when the kit is in use, the analytical reactant becomes associated with the **analyte** of interest via specific binding, the secondary **reagent** being capable of becoming associated with the fluorescent article as a function of the binding of the **analyte** to the specific analytical reactant, a signal from the fluorescent **label** being detectable by a signal detection device independently from the fluorescent signal from the **microparticles**.

USE - The products can be used for detecting **analytes** e.g. an antigen, an antibody, a receptor, a hapten, an enzyme, a protein, a peptide, a nucleic acid, a **drug**, a hormone, a chemical, a polymer, a pathogen, a **toxin**, and combinations (claimed). They can also be used for analyzing an environmental source such as soil, water, or air, or from an industrial source such as taken from a waste stream, a water source, a supply line, a production lot or fermentation media such as from a biological reactor or food fermentation process such as brewing, or foodstuff such as meat, game, produce or dairy products.

Dwg.0/0

L20 ANSWER 23 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-338070 [28] WPIDS
DOC. NO. NON-CPI: N1999-253339
DOC. NO. CPI: C1999-099536
TITLE: Individually detecting **analytes** in a single fluid biological sample.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): EDWARDS, R B; WATKINS, M I
PATENT ASSIGNEE(S): (BIRA) BIO-RAD LAB INC
COUNTRY COUNT: 83
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9926067	A1	19990527	(199928)*	EN	21
AU 9912031	A	19990607	(199943)		

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GD GE GH GM HR HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT UA UG US UZ VN YU ZW

EP 965044 A1 19991222 (200004) EN
R: DE FR GB IT
JP 2000516345 W 20001205 (200067) 26

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9926067	A1	WO 1998-US22812	19981027
AU 9912031	A	AU 1999-12031	19981027
EP 965044	A1	EP 1998-955161	19981027
		WO 1998-US22812	19981027
JP 2000516345	W	WO 1998-US22812	19981027
		JP 1999-519425	19981027

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9912031	A	Based on	WO 9926067
EP 965044	A1	Based on	WO 9926067
JP 2000516345	W	Based on	WO 9926067

PRIORITY APPLN. INFO: US 1997-972563 19971118
AN 1999-338070 [28] WPIDS
AB WO 9926067 A UPAB: 19990719
NOVELTY - A method for individually detecting **analytes** in a single fluid biological **sample** by **assays** that include the binding of species in the biological sample to a solid phase that is in contact with a liquid medium, is new.

DETAILED DESCRIPTION - The method comprises:

(a) using as solid phase a **microparticles** of magnetically responsive material each coupled with an assay **reagent** that is selectively active in an assay for one of the **analytes**, where the **microparticles** varying in size over a range that is an aggregate of subranges, each subrange distinguishable from other subranges of the aggregate by **flow cytometry** and by the assay **reagent** coupled to the **microparticles** of the subrange;

(b) magnetically separating **microparticles** in all of the subranges from the liquid medium; and

(c) defining the liquid medium as a first liquid medium, suspending the **microparticles** separated therefrom in a second liquid medium, analyzing said **microparticles** in said second liquid medium by **flow cytometry** in accordance with the assays.

An INDEPENDENT CLAIM is also included for a composition comprising solid-phase assay **reagents** selectively active

in a plurality of assays each for a different **analyte**, each of the solid-phase assay **reagent** comprising a binding species that is selectively active in a single assay and coupled to one of the **microparticles** of magnetically responsive material, the sizes of said **microparticles** varying in size over a range that is an aggregate of a plurality of subranges, each subrange distinguishable from other subranges of said aggregate by **flow cytometry** and by the binding species coupled thereto.

USE - The method is useful for detecting **analytes** in physiological fluids such as whole blood, serum, urine, spinal fluid, saliva, and stool samples.

Dwg.0/0

L20 ANSWER 24 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1999-312617 [26] WPIDS
DOC. NO. NON-CPI: N1999-233470
DOC. NO. CPI: C1999-092296
TITLE: **Analyte detection** by associating **analyte** in **sample** with scattered-light **detectable particles**.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): JACKSON, J T; KOHNE, D E; YGUERABIDE, E E; YGUERABIDE, J
PATENT ASSIGNEE(S): (GENI-N) GENICON SCI CORP
COUNTRY COUNT: 80
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9920789	A1	19990429	(199926)*	EN	336
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG ZW					
W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB GE GH HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN YU					
AU 9912943	A	19990510	(199938)		
EP 1023456	A1	20000802	(200038)	EN	
R: AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					
CN 1282378	A	20010131	(200131)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9920789	A1	WO 1998-US23160	19981016

AU 9912943	A	AU 1999-12943	19981016
EP 1023456	A1	EP 1998-956415	19981016
		WO 1998-US23160	19981016
CN 1282378	A	CN 1998-812279	19981016

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9912943	A	Based on	WO 9920789
EP 1023456	A1	Based on	WO 9920789

PRIORITY APPLN. INFO: US 1997-953713 19971017

AN 1999-312617 [26] WPIDS

AB WO 9920789 A UPAB: 19990707

NOVELTY - **Analytes** in the sample are specifically associated with a scattered-light detectable **particle**. The **particles** are flowed by an illumination source under conditions to produce scattered light which can be detected by the human eye with less than 500 times magnification and without electronic amplification. The scattered light is detected as a measure of the presence of the **analytes**.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for a method for selection of a **particle** type for detection of an **analyte** by light scattering. Light scattering signal strength versus **particle** size information is provided for candidate **particles**. A **particle** is selected with provides acceptable dynamic range, acceptable low concentration detection sensitivity, and acceptable detection resolution based on this information. These acceptable values and detection resolution are determined by the concentration levels expected for the **analyte** in samples and are sufficient to allow light scattered from the **particles** to be detected by the human eye with less than 500 times magnification and without electronic amplification.

USE - The method may be used for the qualitative or quantitative detection of **analytes** such as industrial or **pharmaceutical compounds** including proteins, peptides, hormones, nucleic acids, lipids, carbohydrates, biological cells and other organisms. It can be adapted to most assay formats commonly used in diagnostic assays.

ADVANTAGE - The high sensitivity and ease-of-use of the signal generation and detection systems, allows the analysis to be effected inexpensively in samples with very low concentrations of the **analyte** without the need to **label** or use electronic amplification. The method can be used for multi-**analyte** detection without the need for complex apparatus.

L20 ANSWER 25 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 2000-037296 [03] WPIDS
CROSS REFERENCE: 1994-303199 [37]; 1996-087113 [09]; 1997-020467 [02]; 1999-069810 [06]; 2000-564407 [41]
DOC. NO. NON-CPI: N2000-027980
DOC. NO. CPI: C2000-009543
TITLE: **Microparticles** useful in diagnostics, therapeutics and research for separations and **drug** delivery as well as in industrial, commercial and cosmetic applications.
DERWENT CLASS: A96 B04 S03
INVENTOR(S): BLIZZARD, C D; BROWN, L R; DI, J; RISKE, F J; SCOTT, T L; SUDHALTER, J; WOISZWILLO, J E
PATENT ASSIGNEE(S): (EPIC-N) EPIC THERAPEUTICS INC
COUNTRY COUNT: 1
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
US 5981719	A	19991109	(200003)*		24

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
US 5981719	A	CIP of	US 1993-28237	19930309
		CIP of	US 1994-206456	19940304
		CIP of	US 1996-699586	19960819
			US 1998-211018	19981214

FILING DETAILS:

PATENT NO	KIND		PATENT NO
US 5981719	A	CIP of	US 5578709

PRIORITY APPLN. INFO: US 1998-211018 19981214; US 1993-28237 19930309; US 1994-206456 19940304; US 1996-699586 19960819
AN 2000-037296 [03] WPIDS
CR 1994-303199 [37]; 1996-087113 [09]; 1997-020467 [02]; 1999-069810 [06]; 2000-564407 [41]
AB US 5981719 A UPAB: 20001023
NOVELTY - **Microparticles** (I), comprising 40 - 100 % by weight (wt%) macromolecules (e.g. immunoglobulins and cell receptor or oligonucleotide probes **labeled** with detectable markers) and polymers in aqueous solution, are new. (I) does not contain any oil.

ACTIVITY - None given.

No biological data given.

MECHANISM OF ACTION - Luteinizing hormone-releasing hormone agonist; luteinizing hormone-releasing hormone antagonist.

USE - (I) may be used in diagnostics, therapeutics and research for separations and **drug** delivery as well as industrial, commercial and cosmetic applications.

They may be used to administer therapeutic proteins or peptides with short half-lives that must be administered by injection. (I) is also used for purification of molecules from complex mixtures or as a **reagent** for detection or quantification of specific molecules or for production of molecules such as antibodies (e.g. in chromatography columns and in immunoaffinity chromatography to separate ligands from complex mixture).

They can be used to detect changes in number of cells or biomolecules in response to particular test conditions using techniques such as **flow cytometry**, used as adjuvants for vaccine production to trigger enhanced immune response for production of antibodies to the antigen in research animals. (I) is also used in cleaning formulations such as formations of enzyme **particles** for addition to detergents, cosmetics (e.g. formations of collagen **particles** to be suspended in lotions or creams), inks and paints.

(I) can be used in in vitro assays such as enzyme-linked immunosorbant assays (ELISA), dot-blot or Western blot for detection of particular targets such as cells, biomolecules or **drugs** in biological sample. Used as visual probes or markers of pathology in histological **samples** for **detection** of rapidly proliferating cells or pathological organisms such as viruses. Used as imaging agents for in vivo localization of molecules, cell types or pathologic conditions for histopathology. Used to detect pathological conditions or to monitor success of therapy, such as chemotherapy or surgery to ensure size of abnormal tissue tumor has decreased or been completely excised (e.g. by X-ray).

Additionally, they may be used for slow-release treatment or prophylaxis of **drugs** such as anti-tumor agents, cytokines, hormones or insulin, directly to site requiring therapy or used as vehicles for gene therapy or production of genetic vaccines when composed of nucleic acids (DNA and RNA) (e.g. for treatment of viruses such as influenza and human immunodeficiency virus (HIV)).

ADVANTAGE - (I) exhibits short- or long-term release kinetics to provide either rapid or sustained release of macromolecules. They allow aqueous fluids to enter and solubilized macromolecules to exit the **microparticle**. Macromolecules released from the **microparticles** retain their natural activity. The polymer they contain removes water from the macromolecules to cause volume exclusion. The **microparticles** are generally uniform in size and shape for injection into patient without **particle**

size selection. Their characteristics may be altered during preparation. Preparation is relatively simple, rapid and inexpensive. Release kinetics of (I) may be manipulated.

DESCRIPTION OF DRAWING(S) - Graph showing cumulative percentage of radiolabeled polyethylene glycol (PEG) and radiolabeled bovine serum albumin (BSA) released from **microparticles** versus square root of time in hours. Black square = polyethylene glycol (PEG); open square = BSA.

Dwg.1/9

L20 ANSWER 26 OF 59 MEDLINE DUPLICATE 3

ACCESSION NUMBER: 1998297767 MEDLINE
DOCUMENT NUMBER: 98297767 PubMed ID: 9635844
TITLE: The effect of 3-week tamoxifen treatment on oestrogen receptor levels in primary breast tumours: a **flow cytometric** study.
COMMENT: Comment in: Br J Cancer. 1999 Mar;79(9-10):1621
AUTHOR: Brotherick I; Browell D A; Shenton B K; Egan M; Cunliffe W J; Webb L A; Lunt L G; Young J R; Higgs M J
CORPORATE SOURCE: Department of Surgery, University of Newcastle upon Tyne, UK.
SOURCE: BRITISH JOURNAL OF CANCER, (1998 May) 77 (10) 1657-60.
Journal code: AV4; 0370635. ISSN: 0007-0920.
PUB. COUNTRY: SCOTLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980713
Last Updated on STN: 20000303
Entered Medline: 19980629

AB The effect of 3-week, preoperative tamoxifen treatment on oestrogen receptor (ER) levels, expressed by primary breast tumours, was examined. Patients (age-matched) with breast cancer, confirmed by fine-needle aspiration, were either treated with 20 mg ml(-1) oral tamoxifen per day or received no medication in the 3-week interval between assessment and surgery. Quantification of ER using **flow cytometry** was performed on the surgically removed tumour samples from tamoxifen-treated (n = 40) and control (n = 38, untreated) patient groups. The tumours were mechanically disaggregated, and saponin treatment rendered these cells permeable to antibodies. Using dual-parameter **labelling** with a FITC-conjugated antibody (NCL-5D3) directed against cytokeratin 8/18/19 and a biotinylated antibody (DAKO-ER 1D5) directed against the oestrogen receptor, ER quantification was determined on a number of receptors per cell basis. Using QC quantum **bead**

standards, ER levels in the epithelial cell population, the non-epithelial cell population and the whole-cell population (ER+) were calculated. ER levels were significantly lower in the total cell population than tamoxifen-treated patients (P = 0.002) when compared with the control (untreated) group. By using a gating procedure using 5D3 antibody positivity, a significantly lower level was **detected** on examining the cytokeratin-positive **population** alone (P = 0.006). Using a complementary gating technique, ER levels were quantified in the cytokeratin-negative cell population. Examination of this group of cells showed no significant difference between the levels of oestrogen receptor found in the tamoxifen-treated and untreated groups (P = 0.4). We have demonstrated that ER levels can be monitored by **flow cytometry**. ER levels in patients treated with tamoxifen 3 weeks before operation are significantly lower than in a comparative group of patients who received no **drug**. Furthermore, the most significant difference in receptor levels is seen by quantification of total ER levels expressed by all the tissue.

L20 ANSWER 27 OF 59 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 1998343132 MEDLINE
DOCUMENT NUMBER: 98343132 PubMed ID: 9678161
TITLE: Immunomagnetic separation and **flow cytometry** for rapid detection of Escherichia coli O157:H7.
AUTHOR: Seo K H; Brackett R E; Frank J F; Hilliard S
CORPORATE SOURCE: Center for Food Safety and Quality Enhancement, Food Science & Technology, University of Georgia, Griffin 30223-1797, USA.
SOURCE: JOURNAL OF FOOD PROTECTION, (1998 Jul) 61 (7) 812-6. Journal code: C48; 7703944. ISSN: 0362-028X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980817
Last Updated on STN: 20000303
Entered Medline: 19980806

AB A rapid method for detecting Escherichia coli O157:H7 combining immunomagnetic **beads** (IMB) and **flow cytometry** was developed. **Labeling** antigens separated by IMB with fluorescent antibody enabled the detection of < 10(3) CFU bacteria per ml in pure culture. The optimum concentration of magnetic **beads** for **flow cytometry** was lower (ca. 10(5) **particles** per ml) than that reported for conventional IMB assay (more than 6 x 10(6) to 8 x 10(6) **particles** per ml). Immunomagnetic separation

and **flow cytometry** (IMFC) were evaluated for detecting E. coli O157:H7 in the presence of a competing microorganism and for detecting antibodies in potassium phosphate buffer. The total assay time from separating antigens with IMB to analyzing with **flow cytometry** was about 1 h. IMFC detected 10(3) to 10(4) CFU of E. coli O157:H7 per ml in ground beef enrichment broth and could effectively discriminate between E. coli O157:H7 and competing natural flora. The new assay system provides another approach to **separation** and **detection** of low **populations** of pathogens and shows potential for detecting low concentrations of **toxins** and other soluble antigens directly from food in a short time.

L20 ANSWER 28 OF 59 MEDLINE
ACCESSION NUMBER: 1998444869 MEDLINE
DOCUMENT NUMBER: 98444869 PubMed ID: 9773885
TITLE: Classification and properties of 64 multiplexed **microsphere** sets.
AUTHOR: Kettman J R; Davies T; Chandler D; Oliver K G; Fulton R J
CORPORATE SOURCE: University of Texas Southwestern Medical Center, Dallas 75235-9048, USA.. kettman@dcaf.swmed.edu
SOURCE: CYTOMETRY, (1998 Oct 1) 33 (2) 234-43.
Journal code: D92; 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990202
Last Updated on STN: 19990202
Entered Medline: 19990119

AB We describe a practical method for the analysis of multiple **analytes** in a single **sample**. The vehicle for each **separate** measurement consists of a set of **microspheres** identifiable by characteristic fluorophores embedded in the **particles**. The use of robust, bench-top **flow cytometers** (flow microfluorimeters) for the analysis of the multiple sets of **microspheres** is facilitated by hardware and software, which acquire the data from the cytometer, classify the **microspheres** according to sets, and collate measurement information from each **microsphere** set in real time. This measurement system can analyze up to 64 **analytes** in a single **sample**. The advantages of multiplexed **assays** using **flow cytometry** include robust measurements, because each **microsphere** set is measured repeatedly. The advantage of the assay's is consistent with simultaneous measurement of many

parameters as well as the speed with which the flow microfluorimeter (cytometer) makes measurements (many hundreds per second). Here, we describe the properties of the **microspheres**, the calibration of the cytometer, and the influence of the properties of the **microspheres** on the sensitivity of measurements.

L20 ANSWER 29 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998140537 EMBASE

TITLE: Magnetic cell separation for purification of human oral keratinocytes: An effective method for functional studies without prior cell subcultivation.

AUTHOR: Formanek M.; Temmel A.; Knerer B.; Willheim M.; Millesi W.; Kornfehl J.

CORPORATE SOURCE: M. Formanek, Department of Otorhinolaryngology, University of Vienna, Wahringer Gurtel 18-20, A-1090 Vienna, Austria

SOURCE: European Archives of Oto-Rhino-Laryngology, (1998) 255/4 (211-215).
Refs: 27
ISSN: 0937-4477 CODEN: EAOTE7

COUNTRY: Germany

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 011 Otorhinolaryngology
029 Clinical Biochemistry

LANGUAGE: English

SUMMARY LANGUAGE: English

AB In studying human oral keratinocytes, it would be very helpful to obtain a pure population of cells without prior in vitro expansion. An immunomagnetic separation technique, or magnetic cell separation (MACS), was modified for efficient purification of human oral keratinocytes. Subsequent to two-step enzymatic digestion, the cell suspension was **labelled** with a mouse anti-CD45 (pan-leukocyte) monoclonal antibody (MoAb) to **stain** mononuclear cells. In a second step a rat anti-mouse antibody conjugated with colloidal superparamagnetic **particles** was used. **Labelled** cells were retained in the magnetic field of a permanent magnet on columns containing a ferromagnetic matrix. The unlabelled, unretained cells were further examined by **flow cytometry** analysis, enzyme-linked immunosorbent assay and polymerase chain reaction. After the MACS procedure, unretained cells showed a strong positivity for the lu-5 MoAb (as a marker for pan-cytokeratin) and were negative for anti-vimentin (to mark mesenchymal cells), for anti-CD45 MoAb and for melanocyte-detecting antibodies, thus representing pure keratinocytes (> 98%). Purified keratinocytes maintained full viability (> 91%) and functional capacities. [3H]thymidine uptake and epidermal growth factor (EGF) receptor expression were unaltered when compared with the non-**separated** cell

population. Furthermore, interleukin-1.alpha. was **detected** at the protein and RNA levels in keratinocytes immediately after MACS enrichment. Our findings show that MACS appears to be a useful tool for purification of oral keratinocytes and allows for further functional studies without prior subcultivation of cells.

L20 ANSWER 30 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1998180346 EMBASE
TITLE: The relationship of HLA-DR, CD38 and CD71 markers to acuvation proliferation and differentiation of some human leukemia and lymphoma cells.
AUTHOR: Glasova M.; Konikova E.; Stasakova J.; Babusikova O.
CORPORATE SOURCE: M. Glasova, Cancer Research Institute, Slovak Academy of Science, 833 91 Bratislava, Slovakia
SOURCE: Neoplasma, (1998) 45/2 (88-95).
Refs: 22
ISSN: 0028-2685 CODEN: NEOLA4
COUNTRY: Slovakia
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We investigated the expression-percentage as well as MESF values ('molecules of equivalent soluble fluorochrom' that represent approximately the density of marker expression) of HLA-DR, CD71 and CD38 markers in some human leukemias (ALL, AML, CLL, CML) and lymphomas. They are non-lineage restricted and are supposed to be activation markers except for cases where they represent pathological phenotype like HLA-DR in pre B-ALL, CD38 in some M0 AML or in plasmocytoma or CD38 and CD71 in less mature T-ALL. We used **flow cytometry**, immunofluorescent **staining** , DNA **staining** by propidium iodide and quantification by calibration **particles**. We demonstrated increased MESF values of HLA-DR compared with controls in all investigated disorders, what could have a prognostic value. We demonstrated significantly higher MESF values of HLA-DR in cALL (37 300 - 46 000) in comparison with AML (9400 - 12 400), what could represent another important parameter when **distinguishing** between these two **groups** of leukemia. In cells of CML patients with lower CD38% and CD71% increased MESF values (5100 for CD38 and 7900 for CD71), were found while in some T-ALL, AML and cALL patients with high percentages of CD71 and CD38 there were lower MESF values what could indicate a possible connection of higher stage of cell maturation with increased density of CD38 and CD71 markers. We investigated possible relationship between percentage of expression of HLA-DR, CD38 and CD71 and proliferation rate by DNA analysis of

the cell cycle. In a group of non-Hodgkin's lymphoma patients, there was no significant increase of proliferation index of malignant cells compared with control. The correlation between percentage of expression of mentioned parameters and proliferation index was not significant. In one patient with Burkitt's lymphoma we demonstrated significant increase of proliferation index of CD71 + subpopulation compared with CD71+ one, what indicates that in aggressive form of NHL CD71 can be evaluated not only as activation but also as proliferation marker.

L20 ANSWER 31 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1997-236023 [21] WPIDS
DOC. NO. NON-CPI: N1997-195101
DOC. NO. CPI: C1997-075754
TITLE: **Bead**-sets for simultaneous assay of multiple **analytes** by cytometric analysis - comprise many subsets carrying specific **reagent** and identifiable from all other subsets by fluorescence parameters, especially for clinical assays, and detecting gene mutation.
DERWENT CLASS: B04 D16 S03 T01
INVENTOR(S): CHANDLER, M B; CHANDLER, V S; FULTON, R J
PATENT ASSIGNEE(S): (LUMI-N) LUMINEX CORP
COUNTRY COUNT: 72
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9714028	A2	19970417	(199721)*	EN	293
RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG					
W: AL AM AT AU BA BB BG CA CH CN CU CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TR TT UA UG US UZ VN					
AU 9673989	A	19970430	(199734)		
US 5736330	A	19980407	(199821)		20
EP 852004	A2	19980708	(199831)	EN	
R: AL AT BE CH DE DK ES FI FR GB GR IE IT LI LT LU LV MC NL PT SE SI					
US 5981180	A	19991109	(199954)		
US 6057107	A	20000502	(200029)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9714028	A2	WO 1996-US16198	19961010

AU 9673989	A		AU 1996-73989	19961010
US 5736330	A		US 1995-542401	19951011
EP 852004	A2		EP 1996-936310	19961010
			WO 1996-US16198	19961010
US 5981180	A		US 1995-540814	19951011
US 6057107	A	Cont of	US 1995-542401	19951011
			US 1998-55329	19980406

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9673989	A	Based on	WO 9714028
EP 852004	A2	Based on	WO 9714028
US 6057107	A	Cont of	US 5736330

PRIORITY APPLN. INFO: US 1995-542401 19951011; US 1995-540814 19951011; US 1998-55329 19980406

AN 1997-236023 [21] WPIDS

AB WO 9714028 A UPAB: 19970522

Preparing a novel **bead**-set which detects many **analytes** in a single fluid sample by **flow cytometric** analysis, comprises: (a) making many **bead** subsets in which the **beads** are sufficiently homogeneous as regards at least 3 classification parameters (CP), but sufficiently different in at least 1 CP from **beads** in all other subsets for the profiles of CP values detected by **flow cytometry** to be unique for each subset; (b) coupling the **beads** of each subset to a reactant specific for **analyte** in a test fluid; and (c) mixing the subsets to produce a **bead**-set in which the subset identity, and thus the nature of coupled reactant, is identifiable from the CP profile.

USE - The method can be used in quantitative and qualitative assay of illicit or therapeutic **drugs**, antigens, (auto)antibodies (particularly immunoglobulin (Ig) belonging to different (sub)classes or those reactive with specific epitopes or allergens, particularly epitopes on an HIV antigen), **analytes** commonly elevated during pregnancy or nucleic acids. The can also be used to determine the epitope to which a monoclonal antibody (MAb) binds. Typical mutations which can be detected by the method of (3) are those in the ret proto-oncogene, low density lipoprotein receptor, adenosine deaminase gene and Duchenne muscular dystrophy gene. The **bead**-set is also used to construct a multiplexed standard assay for use in quantification.

ADVANTAGE - The **bead**-set provides simultaneous and automated determination and interpretation of numerous **analytes** in a single step, and in real time, reducing the

costs of diagnostic and genetic analyses.
Dwg.27/51

L20 ANSWER 32 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97238387 EMBASE
DOCUMENT NUMBER: 1997238387
TITLE: Transmission of HIV-1 in infants born to seropositive mothers: Pcr- amplified proviral DNA detected by **flow cytometric** analysis of immunoreactive **beads**.
AUTHOR: Dorenbaum A.; Venkateswaran K.S.; Yang G.; Comeau A.M.; Wara D.; Vyas G.N.
CORPORATE SOURCE: G.N. Vyas, UCSF, Box 0134, Univ. of California Sch. of Med., San Francisco, CA 94143, United States
SOURCE: Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology, (1997) 15/1 (35-42).
Refs: 23
ISSN: 1077-9450 CODEN: JDSRET
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The diagnosis of HIV infection in newborns is established by amplification of proviral DNA using the polymerase chain reaction (PCR). We developed a non-isotopic method for heminested PCR using a biotinylated primer among sets of three oligonucleotides, each selected from the HIV long terminal repeat (LTR) and gag sequences. An internal probe incorporating digoxigenin-dUTP was also synthesized by PCR. The PCR products, hybridized with LTR region or gag region probes, were captured with streptavidin-**coated** magnetic **beads** and detected by fluorescein isothiocyanate-**labeled** antidigoxigenin in **flow cytometric** analysis. This immunoreactive **bead** assay (PCR-IRB) detected about three copies of HIV proviral DNA. A panel of 50 coded DNA **specimens** of infants previously **assayed** by conventional PCR and with known clinical results revealed that the PCR-IRB findings using LTR, but not gag, were in agreement. A double-blind prospective study of blood samples from 14 mother-infant pairs using the PCR-IRB amplification of LTR gave results similar to the commercial Amplicor HIV-1 PCR test and were consistent with the clinical outcomes. PCR-IRB results were positive for 11 mothers and three infants, one at birth, one at 2 weeks after birth, and one at 8 weeks after birth. PCR-IRB is a simple, reliable, specific, and automatable assay useful in the early diagnosis of perinatal HIV infection in clinical practice and regional screening programs.

L20 ANSWER 33 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97028686 EMBASE
DOCUMENT NUMBER: 1997028686
TITLE: Isolation and phenotypic characterization of CD117-positive cells.
AUTHOR: Neu S.; Geiselhart A.; Kuci S.; Baur F.; Niethammer D.; Handgretinger R.
CORPORATE SOURCE: S. Neu, Universitats-Kinderklinik Tubingen, Forschungsabt Padiatr Hamatol Onkol, Rumelinstr 23, D-72070 Tubingen, Germany. KKrhand@uni-tuebingen.de
SOURCE: Leukemia Research, (1996) 20/11-12 (963-971).
Refs: 27
ISSN: 0145-2126 CODEN: LEREDD
PUBLISHER IDENT.: S 0145-2126(96)00077-X
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Mononuclear cells derived from cord blood were **stained** using the CD117-specific, fluorochrome-**labeled** monoclonal mouse antibody 95C3. Additional **staining** was performed using an isotype-specific rat-anti-mouse antibody, **labeled** with supermagnetic **microparticles**. Target cells were enriched by the technique of magnetic cell **separation**, MACS. The resulting cell **population** contained 96.5% (.+-.1.7% S.D.) CD117-expressing cells (n = 12) with different levels of CD117 antigen expression. Using **flow cytometry**, two cell populations differing in size were found. A majority (93%) of cells with high forward scatter revealed a phenotype positive for CD117 and CD34. Isolated cells revealed a high fraction of hematopoietic progenitors (16%). The technique presented allows for an alternative approach of stem cell enrichment and might be useful in autologous transplantation of cells with hematopoietic properties.

L20 ANSWER 34 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97034458 EMBASE
DOCUMENT NUMBER: 1997034458
TITLE: Apoptosis of cells in aged **samples** as **detected** by the ProCOUNT **reagent**.
AUTHOR: Manion K.; Frey T.
CORPORATE SOURCE: T. Frey, BDIS, 2350 Qume Dr., San Jose, CA 95131, United States
SOURCE: Communications in Clinical Cytometry, (1996) 26/4 (317-322).

Refs: 16
ISSN: 0196-4763 CODEN: CCCYEM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB An unusual population of high side scatter, low nucleic acid **dye** binding, dim CD45 cells was found in aged blood samples **stained** with the ProCOUNT **reagent**. Cell surface **staining** showed that these cells have the surface phenotype of neutrophils. However, they have decreased expression of several surface antigens, bind annexin V, and **stain** more dimly than normal neutrophils with LDS-751. These characteristics indicate that the cells have become apoptotic. The decreased expression of the CD45 antigen on apoptotic neutrophils could have an impact on some methods for enumerating CD34-positive progenitor cells. Absolute CD34-positive cell counts are frequently obtained by multiplying CD34-positive cells as a percentage of the total cells by the white blood cell count from a hematologyanalyzer. Cells **staining** dimly with CD45 may not pass a **flow cytometer** threshold set on this parameter but appear to be detected by a hematology instrument thresholding on cell size. Thus the white blood cell counts from the two sources may not be identical, introducing error into the calculated absolute CD34 count. Absolute counts of CD34 cells based on simultaneous acquisition of a counting **bead** are not affected by the presence of this population, but purity estimates can be affected if the possible presence of these cells is not considered in aged samples.

L20 ANSWER 35 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1995-194210 [25] WPIDS
DOC. NO. NON-CPI: N1995-152410
DOC. NO. CPI: C1995-089902
TITLE: Counting of progenitor cells in samples - by **flow cytometry** using a nucleic acid **dye**, MAb which reacts with leukocytes, CD34 antibody and fluorescent **beads**.
DERWENT CLASS: B04 D16 J04 S03
INVENTOR(S): CHEN, C; TERSTAPPEN, L
PATENT ASSIGNEE(S): (BECT) BECTON DICKINSON CO
COUNTRY COUNT: 17
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 9513540 A1 19950518 (199525)* EN 28
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: JP
EP 685071 A1 19951206 (199602) EN
R: DE ES FR GB IT NL
JP 08501396 W 19960213 (199643) 29
JP 2680931 B2 19971119 (199751) 10

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9513540	A1	WO 1994-US12952	19941110
EP 685071	A1	WO 1994-US12952	19941110
		EP 1995-901205	19941110
JP 08501396	W	WO 1994-US12952	19941110
		JP 1995-513998	19941110
JP 2680931	B2	WO 1994-US12952	19941110
		JP 1995-513998	19941110

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 685071	A1	Based on	WO 9513540
JP 08501396	W	Based on	WO 9513540
JP 2680931	B2	Previous Publ.	JP 08501396
		Based on	WO 9513540

PRIORITY APPLN. INFO: US 1993-151086 19931112
AN 1995-194210 [25] WPIDS
AB WO 9513540 A UPAB: 19950630
Counting the absolute number of progenitor cells (PCs) in a sample comprises: (a) **labelling** cells in a sample with a **compsn**. comprising (i) a nucleic acid **dye** that will selectively react with nucleated cells, (ii) a monoclonal antibody (MAb), **labelled** with a first fluorochrome, that will selectively react with mature lymphoid, neutrophil, erythroid and monocytic cells and weakly with PCs, (iii) a CD34 antibody **labelled** with a second fluorochrome and (iv) a known number of fluorescent **beads**; and (b) analysing the **labelled** cells by **flow cytometry** comprising (i) setting a fluorescence threshold on the fluorescence emitted by the nucleic acid **dye** so as to include all nucleated cells and **beads**, (ii) analysing the cells and **beads** in the sample that meet or exceed the threshold for light scatter and fluorescence emissions, (iii) using fluorescence emissions and scatter data recorded to discriminate between and

among the various cell leukocyte populations in the sample and **beads** and (iv) counting the number of **beads** and CD34+ cells and determining the ratio.

USE - The method is used partic. for determining the number of CD34+ cells in a sample and subsets of CD34+ cells such as CD34+/CD38-/HLA-DR+ cells.

ADVANTAGE - The method can provide a rapid and accurate **determn**. of PCs in a **sample**.

Dwg.0/6

L20 ANSWER 36 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95206250 EMBASE
DOCUMENT NUMBER: 1995206250
TITLE: Lymphocyte **subset determination** using a hematology analyzer.
AUTHOR: Hudson J.C.; Brunhouse R.F.; Garrison C.; Rodriguez C.M.; Zwerner R.; Russell T.R.
CORPORATE SOURCE: Coulter Corporation, 21A01, P.O Box 169015,Miami, FL 33116-9015, United States
SOURCE: Communications in Clinical Cytometry, (1995) 22/2 (150-153).
ISSN: 0196-4763 CODEN: CCCYEM
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
006 Internal Medicine
025 Hematology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Anti-CD4 antibody (T4)-**coated microspheres** were used to **label** CD4 cells in whole blood. The mixture was lysed and analyzed by a modified Coulter VCS hematology analyzer, which differentiated **microsphere-labeled** cells by a change in Coulter volume, conductance, and light scatter. %CD3+/CD4+ fluorescent values from a profile were compared to %CD4 values using the VCS-**microsphere** method. CD3 gating was used to exclude CD4+ monocytes from the 90LS-FALS lymphocyte gate. The results correlated well (R = 0.996). The percentage of CD4+ lymphocytes from profile scatterplots and VCS scatterplots showed a line of regression close to the equivalence line (n = 76, slope = 0.96) when CD3 gating was used for the profile. These results suggest that CD3 gating, though necessary for 90LS-FALS scatterplots, may not be necessary for volume-conductance-light scatterplots.

L20 ANSWER 37 OF 59 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 95130947 MEDLINE

DOCUMENT NUMBER: 95130947 PubMed ID: 7530268
TITLE: Dual **analyte** assay based on **particle** types of different size measured by **flow cytometry**.
AUTHOR: Frengen J; Lindmo T; Paus E; Schmid R; Nustad K
CORPORATE SOURCE: Department of Physics, University of Trondheim, Norway.
SOURCE: JOURNAL OF IMMUNOLOGICAL METHODS, (1995 Jan 13) 178 (1) 141-51.
Journal code: IFE; 1305440. ISSN: 0022-1759.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199502
ENTRY DATE: Entered STN: 19950307
Last Updated on STN: 19960129
Entered Medline: 19950217

AB Simultaneous **flow cytometric** assays have been developed for alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG), with internal **determination** of **sample** related non-specific binding (NSB). The assays use **particles** of 7.5, 6.5 and 5.5 microns diameter **coated** with, respectively, monoclonal antibodies specific for AFP, hCG or an epitope normally not present in serum. The different **particle** types were identified simultaneously by light-scatter measurements as their specific immunofluorometric responses were determined. The NSB in the simultaneous assay of AFP and hCG was increased by approximately 30% compared to corresponding single **analyte** assays. The working range of the dual **analyte** assays was 0.6-2000 kIU/l for AFP and 6-10,000 IU/l for hCG. No significant interference from the presence of the other **analyte** was observed in the measurement of either AFP or hCG. The 95% confidence interval for the ratio of dual over single **analyte** assay results was [0.81, 1.11] for AFP and [0.88, 1.16] for hCG.

L20 ANSWER 38 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 95218715 EMBASE
DOCUMENT NUMBER: 1995218715
TITLE: Determination of CD4+ and CD8+ lymphocytes with the **cytosphere** assay: A comparative study with **flow cytometry** and the immunoalkaline phosphatase method.
AUTHOR: Gernow A.; Lisse I.M.; Bottiger B.; Christensen L.; Brattegaard K.
CORPORATE SOURCE: Department of Pathology, Hvidovre University Hospital,Copenhagen, Denmark

SOURCE: Clinical Immunology and Immunopathology, (1995) 76/2 (135-141).
ISSN: 0090-1229 CODEN: CLIIAT
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The proportion and absolute numbers of CD4+ and CD8+ lymphocytes in peripheral blood were determined using a new manual method, the **cytosphere** assay (CA). This method uses small latex **beads coated** with monoclonal antibodies directed against the CD4 and CD8 receptors, respectively. The CA was compared with two other methods for **determination** of T lymphocyte **subsets**, **flow cytometry** (FC) and the immunoalkaline phosphatase (IA) method, by testing HIV-seropositive and HIV-seronegative samples from Denmark (44) and Ivory Coast (79). For HIV-seropositive samples, both the proportion and the absolute number of CD4+ lymphocytes determined by CA showed a good correlation with results obtained by FC (correlation coefficients were 0.92 and 0.74 in Denmark and The Ivory Coast, respectively) and IA (correlation coefficients were 0.94 and 0.66 in Denmark and The Ivory Coast, respectively). However, for HIV-seronegative samples the corresponding correlation coefficients were low. CD4% determinations deviated more from FC counts at higher CD4 counts than at lower levels for both seronegative and seropositive individuals. In conclusion, the CA performed best for samples from HIV-infected individuals. Before a more general utilization of the method, it is necessary to improve its repeatability and standardize its performance at all levels of CD4+ T cells.

L20 ANSWER 39 OF 59 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 95324309 MEDLINE
DOCUMENT NUMBER: 95324309 PubMed ID: 7541332
TITLE: Use of the biotinylated antibody DAKO-ER 1D5 to measure oestrogen receptor on cytokeratin positive cells obtained from primary breast cancer cells.
AUTHOR: Brotherick I; Lennard T W; Cook S; Johnstone R; Angus B; Winthereik M P; Shenton B K
CORPORATE SOURCE: Department of Surgery, Medical School, University of Newcastle, Denmark.
SOURCE: CYTOMETRY, (1995 May 1) 20 (1) 74-80.
Journal code: D92; 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950822
Last Updated on STN: 19970203
Entered Medline: 19950804

AB A method for the use of a biotinylated antibody (DAKO-ER 1D5) to quantify oestrogen receptors (ER) on tumour cells by **flow cytometry** is described. ER quantification was determined after treatment with saponin rendering cells permeable to ER antibody. Use of dual parameter **labelling** was performed utilizing a FITC-conjugated antibody (NCL-5D3) directed against cytokeratin 8/18. This allowed selection of breast cancer cells of epithelial origin by gating to exclude contaminating inflammatory and stromal cells. Use of such a gating technique was seen to identify cells with a higher level of ER expression. Using QC quantum **bead** standards, the number of ER binding sites per cell was assessed. Results were compared with conventional ER quantification using a radio-ligand binding assay. A high degree of correlation was found between the two methods. The **flow cytometric** method for ER quantification described is simple, rapid, and reproducible. The assay may be of particular value in measuring ER on urgent clinical **samples**. Advantages of this **assay** over the radio-ligand binding assay include reduction in use of radio-**labelled** iodine **compounds**, a decrease in analysis time, and reduced cost and quantity of material needed for assay.

L20 ANSWER 40 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94355633 EMBASE
DOCUMENT NUMBER: 1994355633
TITLE: Expression of CD4 by human hematopoietic progenitors.
AUTHOR: Louache F.; Debili N.; Marandin A.; Coulombel L.; Vainchenker W.
CORPORATE SOURCE: INSERM U.362, Institut Gustave Roussy,94805 Villejuif, France
SOURCE: Blood, (1994) 84/10 (3344-3355).
ISSN: 0006-4971 CODEN: BLOOAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
025 Hematology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB It has been recently reported that murine hematopoietic stem cells and progenitors express low levels of CD4. In this study, we have investigated by phenotypic and functional analysis whether the CD4 molecule was also present on human hematopoietic progenitors. Unfractionated marrow cells or immunomagnetic **bead**

-purified CD34+ cells were analyzed by two-color fluorescence with an anti-CD4 and an anti-CD34 monoclonal antibody (MoAb). A large fraction (25% to 50%) of the CD34+ cells was weakly **stained** by anti- CD4 antibodies. Moreover, in further experiments analyzing the expression of CD4 in different subpopulations of CD34+ cells, we found that CD4 was predominantly expressed in phenotypically primitive cells (CD34+ CD38(- /low) CD71(low) Thy-1(high), HLA-DR(+/low)). However, the presence of CD4 was not restricted to these primitive CD34+ cell **subsets** and was also **detected** in a smaller fraction of more mature CD34+ cells exhibiting differentiation markers. Among those, subsets with myelo-monocytic markers (CD13, CD33, CD14, and CD11b) have a higher CD4 expression than the erythroid or megakaryocytic subsets. In vitro functional analysis of the **sorted** CD34+ **subsets** in colony **assays** and long-term culture-initiating cell (LTC-IC) assays confirmed that clonogenic progenitors (colony-forming unit-granulocyte-macrophage, burst-forming unit-erythroid, and colony-forming unit-megakaryocyte) and LTC-IC were present in the CD4(low) population. However, most clonogenic progenitors were recovered in the CD4- subset, whereas the CD4(low) fraction was greatly enriched in LTC-IC. In addition, CD4(low) LTC-IC generated larger numbers of primitive clonogenic progenitors than did CD4- LTC-IC. These observations suggest that, in the progenitor compartment, the CD4 molecule is predominantly expressed on very early cells. The CD4 molecule present on CD34+ cells appeared identical to the T-cell molecule because it was recognized by three MoAbs recognizing different epitopes of the molecule. Furthermore, this CD4 molecule is also functional because the CD34+ CD4(low) cells are able to bind the human immunodeficiency virus (HIV) gp120. This observation might be relevant to the understanding of the mechanisms of HIV- induced cytopenias.

L20 ANSWER 41 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94220500 EMBASE
DOCUMENT NUMBER: 1994220500
TITLE: Heterogeneity of endosomal populations in the rat renal cortex: Light endosomes.
AUTHOR: Hammond T.G.; Verroust P.J.
CORPORATE SOURCE: H4/510 Clinical Science Center, Univ. of Wisconsin Medical School, 600 Highland Ave.,Madison, WI 53792, United States
SOURCE: American Journal of Physiology - Cell Physiology, (1994) 266/6 35-6 (C1783-C1794).
ISSN: 0363-6143 CODEN: AJPCDD
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 002 Physiology
037 Drug Literature Index

LANGUAGE: English
SUMMARY LANGUAGE: English

AB The endosomal pathway of the rat renal cortex was **labeled** by intravenous infusion of fluorescent dextran small enough to cross the glomerular ultrafiltration barrier and be taken up by luminal endocytosis in the proximal tubule. Using Percoll gradient centrifugation, we isolated and characterized a previously undetected renal cortical endosomal fraction slightly lighter than basolateral membranes. Assay of entrapped dextran on a vesicle-by-vesicle basis using small-**particle flow cytometry** techniques demonstrates homogeneity for entrapped dextran. **Flow cytometry** colocalization of entrapped markers with brush-border enzymes in >99% of the vesicles and the absence of Na-K-adenosinetriphosphatase (ATPase) suggest both that these vesicles are of apical origin and that apical enzymes traffic into endosomal elements. Furthermore, two glycoproteins derived from intermicrovillar clefts are detectable in this fraction. Ultrastructurally the vesicles are heterogeneous, consisting of multivesicular bodies together with vesicles of diverse size and **coating**. **Populations** of vesicles can be cleanly **separated** from each other and basolateral membranes according to their surface charge by high-resolution free-flow electrophoresis. Multiparameter **flow cytometry** analysis demonstrates that a more abundant population of smaller vesicles has brisker H+-ATPase activity, whereas a less abundant population of larger vesicles has slower H+-ATPase activity. In contrast, brush-border membrane vesicles contained no entrapped markers and lacked H+-ATPase activity. Analysis of vesicles prepared after addition of dextran to the homogenate confirms that the vesicles form in vivo. Hence a heterogeneous renal cortical endosomal population is of apical origin.

L20 ANSWER 42 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94245840 EMBASE
DOCUMENT NUMBER: 1994245840
TITLE: Biological properties of subpopulations of pluripotent hematopoietic stem cells enriched by elutriation and **flow cytometry**.
AUTHOR: Orlic D.; Anderson S.; Bodine D.M.; Quesenberry P.J.; Gearing; Bernstein I.D.; Adamson J.W.; Terstappen L.W.M.M.; Yoder
CORPORATE SOURCE: Laboratory of Gene Transfer, National Human Genome Research Ctr., NIH, 9000 Rockville Pike,Bethesda, MD 20892, United States
SOURCE: Blood Cells, (1994) 20/1 (107-120).
ISSN: 0340-4684 CODEN: BLCEDD
COUNTRY: United States

DOCUMENT TYPE: Journal; Conference Article
FILE SEGMENT: 025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We have studied several features of pluripotent hematopoietic stem cells (PHSCs) and day-12 spleen colony-forming units (CFU-S) obtained from adult murine bone marrow. Single-cell suspensions of C57BL/6J mouse bone marrow were fractionated by counterflow centrifugal elutriation at flow rates (FR) of 15, 25, 30, and 35 ml/min, and with the rotor off (R/O). The fractions FR25 and FR35 contained approximately equal numbers of PHSC that could repopulate W/W(v) mice. These PHSCs were further enriched by subtracting lineage-positive cells using monoclonal antibodies (MAb) and magnetic **immunobeads**. The resulting lineage-negative cells (Lin-) were then **stained** with a MAb for the c-kit receptor and **sorted** by **flow cytometry**. Both **subsets** were fractionated into cells expressing high (bright) (c-kit(BR)), low (dull) c-kit(DULL) and no (negative, c-kit(NEG)) c-kit receptor. As few as 100 to 200 c-kit(BR) cells could repopulate the entire thymus and bone marrow in W/W(v) mice. No PHSCs were present in the c-kit(DULL) and c-kit(NEG) fractions. We assayed fresh bone marrow and elutriation fractions FR25 and FR35 for gene expression by reverse transcriptase polymerase chain reaction. Using a semiquantitative protocol, we detected mRNA for .beta.-globin and flk-2, a protein tyrosine kinase receptor, in all samples except the FR25 Lin- c- kit(BR) subset. We consider the cells in FR25 Lin- c-kit(BR) to be the most primitive set of hematopoietic stem cells.

L20 ANSWER 43 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94092146 EMBASE
DOCUMENT NUMBER: 1994092146
TITLE: An immunomagnetic separation method using superparamagnetic (MACS) **beads** for large-scale purification of human mammary luminal and myoepithelial cells.
AUTHOR: Clarke C.; Titley J.; Davies S.; O'Hare M.J.
CORPORATE SOURCE: The Institute of Cancer Research, Haddow Laboratories, 15 Cotswold Road,Sutton, Surrey SM2 5NG, United Kingdom
SOURCE: Epithelial Cell Biology, (1994) 3/1 (38-46).
ISSN: 0940-9912 CODEN: ECBIEP
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB A comparison has been made between different immunomagnetic

techniques for separating normal human mammary epithelial cells based on the exclusive expression of EMA (epithelial membrane antigen) by luminal cells and CALLA (CD10) by myoepithelial cells. When cells **labelled** with antibodies to these antigens were incubated with **Dynabeads**, they rosetted myoepithelial but not luminal cells. However, both luminal and myoepithelial cells could be positively separated using the MACS system. Purity was established by analyzing the expression of CALLA and EMA using **flow cytometry**, and of cell-type specific cytokeratins using indirect immunofluorescence. **Dynabead-separated** myoepithelial cell **populations** were of high purity (>98%) but the **beads** could not be removed from the cells. Luminal cell **populations separated** by the MACS method were also highly purified (>95%), as were myoepithelial cell **populations** (>90%). Using this immunomagnetic **separation** method, up to 107 cells of each type could be obtained from individual preparations.

L20 ANSWER 44 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1993-058913 [07] WPIDS
DOC. NO. NON-CPI: N1993-044857
DOC. NO. CPI: C1993-026379
TITLE: Simultaneous analysis of multiple **analytes** - esp. for application to screens and panels, without use of multiple fluorescers and detectors.
DERWENT CLASS: B04 C07 D16 J04 S03 S05 T01
INVENTOR(S): CROTHERS, S D; LEHNEN, B C
PATENT ASSIGNEE(S): (TRAN-N) TRANSMED BIOTECH INC; (TRAN-N) TRANS-MED BIOTECH INC
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9302360	A1	19930204	(199307)*	EN	61
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL SE					
W: AU CA JP NO US					
AU 9223480	A	19930223	(199324)		
EP 594763	A1	19940504	(199418)	EN	
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL SE					
JP 06509417	W	19941020	(199501)		21
EP 594763	A4	19960626	(199644)		
US 5567627	A	19961022	(199648)		15
EP 594763	B1	19980923	(199842)	EN	
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL SE					
DE 69227112	E	19981029	(199849)		
CA 2113350	C	19990323	(199930)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 9302360	A1		WO 1992-US5799	19920710
AU 9223480	A		AU 1992-23480	19920710
EP 594763	A1		EP 1992-916006	19920710
			WO 1992-US5799	19920710
JP 06509417	W		WO 1992-US5799	19920710
			JP 1993-502870	19920710
EP 594763	A4		EP 1992-916006	
US 5567627	A	Cont of	US 1991-731039	19910716
			US 1993-149129	19931105
EP 594763	B1		EP 1992-916006	19920710
			WO 1992-US5799	19920710
DE 69227112	E		DE 1992-627112	19920710
			EP 1992-916006	19920710
			WO 1992-US5799	19920710
CA 2113350	C		CA 1992-2113350	19920710

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9223480	A	Based on	WO 9302360
EP 594763	A1	Based on	WO 9302360
JP 06509417	W	Based on	WO 9302360
EP 594763	B1	Based on	WO 9302360
DE 69227112	E	Based on	EP 594763
		Based on	WO 9302360

PRIORITY APPLN. INFO: US 1991-731039 19910716; US 1993-149129 19931105

AN 1993-058913 [07] WPIDS

AB WO 9302360 A UPAB: 19931119

Method for contiguously detecting multiple **analytes** (I) of interest in a sample comprises (a) combining the sample with a **compsn**. comprising known proportions of multiple discrete subpopulations of **reagents** capable of binding specifically to (I) to form a discrete population of specific binding pairs (Sbps) for each (I), (the **compsn**. is claimed more generally as a **reagent** comprising a mixt. of multiple discrete ligand complexes, each of known proportion in the **compsn**.), the subpopulations being linked to particulate supports which may be detected by **flow cytometry** techniques and not separately identifiable based upon physical characteristics of the particulate supports; (b) contacting the sbp's with an agent **labelled** with a fluorochrome which

eits a detectable fluorescence on exposure to excitation energy, the agent being capable of binding to the **reagents**; (c) detecting fluorescence intensity of a preselected number of the particulate supports using **flow cytometry** techniques, a partic. fluorescence intensity being obtd. from each discrete population of sbp's and (d) relating the presence of (I) to the formation of sbp's associated with each of the known proportions of multiple subpopulations of **reagents** by comparing the area of peaks in the fluorescence histograms total area of peaks in the histogram.

(1) Analysing histograms using a computer comprises (i) providing one or more histogram data as input data to the computers (ii) removing one or more nonessential feature data from the input data to generate an enhanced input data; (iii) extracting one or more topographical feature data from the enhanced input data to define a set of data markers; (iv) identifying one or more topographical regions in the enhanced input data corresp. to the set of data markers; (v) calculating areas and means of each of the identified topographical regions; (vi) correlating the regions to one or more assay **analytes** on the basis of the results of (v); and (vii) providing an assay data report as an output. (2) Analysing multiplex histograms including **cpd**. and overlapping peaks by (i) entering into a computer several histograms; (ii) smoothing the histograms with a 5 point Ganssian smooth method so that nonessential features are removed; (iii) extracting features by locating peaks, valleys and plains in the smoothed histogram; (iv) dividing the smoothed histograms into regions based on the valleys and plains; (v) editing the peaks by suppressing debris and aggregate peaks and suppressive markers set excessively high above background; (vi) calculating areas and means of regions; (vii) assigning **analytes** to designated regions by calculating area ratios and using a look-up table for assignment of regions to assay **analytes**; (viii) reporting assay means by correlating mcf to assay **analytes**; and (ix) outputting the results.

USE/ADVANTAGE - The methods greatly extend the number of contiguous analyses which may be performed on a single **sample** and fluid partic. applicn. in **screens**, panels and combination tests of all types. At least five **analytes** can be identified in a single assay without the use of discriminators, such as changes in **bead** size or the use of multiple fluorescers and detectors.

0/0

Dwg.0/0

ABEQ US 5567627 A UPAB: 19961202

A method for simultaneously detecting multiple **analytes** of interest in a sample, said method comprising:

(a) combining said sample with a composition comprising a

population of particulate supports that are **detectable** by **flow cytometry** techniques, wherein

A. each particulate support consists essentially of an unlabelled **particle** to which is bound exactly one of a set of at least two unlabelled specific **reagents**, each of said specific **reagents** is capable of binding specifically to one of the multiple **analytes** of interest,

B. the population comprises discrete subpopulations of supports, each of which consists of those supports comprising the same specific **reagent**, wherein the combined number of supports in any two or more subpopulations is unique as compared to the number of supports in any other combination of subpopulations or as to a single subpopulation,

C. each subpopulation constitutes a predetermined, known proportion of the population of particulate supports, and

D. the **particles** of the population are substantially physically indistinguishable from each other, the particulate supports being of approximately the same mean diameter, whereby in the presence of one or more **analytes** a discrete population of specific binding pars is formed on the supports of each subpopulation for each **analyte** of interest in the sample;

(b) contacting said specific binding parts with a **labelled** agent, said **labelled** agent comprising a number of specific binding moieties each attached to a fluorochrome which emits a detectable fluorescence upon exposure to excitation energy, wherein each of the binding moieties is specific for one of the multiple **analytes** and the same fluorochrome is attached to each of the binding moieties;

(c) removing any unbound **labelled** agent and detecting fluorescence intensity of each particulate support of a preselected number of said population of particulate supports using **flow cytometry** techniques;

(d) obtaining a histogram plot of said preselected number of particulate supports detected as a function of the logarithm of said fluorescence intensity detected, said histogram plot contains one or more peaks, wherein each peak has an area which indicates the proportion of each subpopulation of particulate supports of said preselected number associated with said peak, and wherein each peak has a position for each **analyte** detected which may be the same as or different from the peak position of any other **analyte** detected and wherein absence of one or more **analytes** results in a peak having a position at essentially background fluorescence; and

(e) identifying **analytes** present in said simple by the relative proportion of each peak as a function of the relative proportion of said preselected supports.

Dwg.0/6

L20 ANSWER 45 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93290375 EMBASE
DOCUMENT NUMBER: 1993290375
TITLE: Preparation of cell nuclei from fresh tissues for high-quality DNA **flow cytometry**.
AUTHOR: Castro J.; Heiden T.; Wang N.; Tribukait B.
CORPORATE SOURCE: Dept. of Medical Radiobiology, Karolinska Institute, Box 60212,S-10401 Stockholm, Sweden
SOURCE: Cytometry, (1993) 14/7 (793-804).
ISSN: 0196-4763 CODEN: CYTODQ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
025 Hematology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB An easy method for preparation of bare cell nuclei from fresh solid tissues for DNA **flow cytometry** is described.
Pieces of up to 2 x 2 x 2 mm3 size from fresh tissues were fixed in formalin. After removal of formalin by washing with ethanol and rehydration with tap water, the tissue pieces were incubated with subtilisin Carlsberg (pronase, Sigma protease XXIV) and then **stained** directly with DAPI. **Staining** with ethidium bromide gave unsatisfactory results. Neither mechanical disaggregation nor centrifugation were used. The resulting cell nucleus suspensions had extremely low frequencies of debris **particles** and of clumped cell nuclei. A good yield, a minimized loss, and a good **stainability** of cell nuclei were obtained. The applicability of the method was exemplified by the analysis of biopsies from the colon-rectum in patients with ulcerative colitis and of biopsies from the bladder in patients with bladder cancer and compared to the standard method of this laboratory, which uses mechanical disaggregation, ethanol fixation, pepsin treatment, and **staining** with ethidium bromide. The formalin-subtilisin Carlsberg technique resulted in good agreement of ploidy measurements compared to the standard method, a higher number of evaluable histograms, an improved **detectability** of aneuploid cell **populations**, and an improved accuracy of the S- and G2-phase analysis, particularly in samples with low proliferation. The method also makes it possible to use long-term storage and to transport samples by post.

L20 ANSWER 46 OF 59 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 93358471 MEDLINE
DOCUMENT NUMBER: 93358471 PubMed ID: 8394789
TITLE: GABAergic cells and signals appear together in the early post-mitotic period of telencephalic and

striatal development.

AUTHOR: Fiszman M L; Behar T; Lange G D; Smith S V; Novotny E A; Barker J L

CORPORATE SOURCE: Laboratory of Neurophysiology, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

SOURCE: BRAIN RESEARCH. DEVELOPMENTAL BRAIN RESEARCH, (1993 Jun 8) 73 (2) 243-51.
Journal code: DBR; 8908639. ISSN: 0165-3806.

PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199309

ENTRY DATE: Entered STN: 19931008
Last Updated on STN: 19970203
Entered Medline: 19930922

AB Single cell suspensions derived from embryonic telencephala taken from embryos of gestational day 13 (E13) as well as rat striatal tissue from E14, 15 and 17 were prepared by tissue digestion with papain. Cell suspensions were analyzed by **flow cytometry** or plated onto poly-D-lysine-**coated** culture dishes for either nuclear **staining** or immunocytochemistry. Experiments on functional Na+ channels and GABAA receptor expression were carried out using a fluorescence-activated cell sorter (FACS) and a negatively charged fluorescent indicator **dye** (oxonol). FACS analysis of embryonic cell suspensions at E13-17 consistently revealed one major subpopulation accounting for 85-90% of the events and one minor subpopulation (10-15% of the total). When **sorted**, the major **subpopulation** consisted of phase-bright cells of 5-7 microns diameter some of which had neurites. The minor population consisted of phase-dark cells and resealed membranes of 0.5-4 microns diameter as well as debris. Almost all the cells obtained in the high FALS (forward-angle light scatter) subpopulation at E17 expressed 200-kDa neurofilament and tetanus **toxin** antigens while the small diameter cells seldom expressed tetanus **toxin** and **particles** never did. A small number of GABA-containing neurons were detected in the telencephalon at E13 (3%) and in the developing striatum at E14 (6%). All of the GABA-containing neurons expressed neurofilament. In the embryonic rat striatum, nanomolar concentrations of muscimol (GABAA agonist) induced depolarizing responses. A small number of cells in the high FALS subpopulation were responsive to muscimol starting at embryonic day 14, and the number of responsive cells increased at E15.(ABSTRACT TRUNCATED AT 250 WORDS)

L20 ANSWER 47 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 93316852 EMBASE
DOCUMENT NUMBER: 1993316852
TITLE: Polymorphonuclear leucocyte function tests: A comparison of cytochrome C reduction and **flow cytometric** analysis.
AUTHOR: Matsuda J.; Tsukamoto M.; Saitoh N.; Gohchi K.; Jimbo S.; Shibui T.; Moriya K.
CORPORATE SOURCE: Department of Medicine, Teikyo University School of Medicine, 11-1, Kaga 2-Chome,Itabashi-Ku, Tokyo 173, Japan
SOURCE: British Journal of Biomedical Science, (1993) 50/1 (60-63).
ISSN: 0967-4845 CODEN: BJMSEO
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Assay results were compared between the cytochrome C reduction test and **flow cytometry** using fluorescent **beads** or DCFH-DA (2', 7'-dichlorofluorescein diacetate) granules, in examining the phagocytosing and killing properties of polymorphonuclear leucocytes (PMN). When PMN **samples** from 20 healthy persons were **assayed**, no correlation was found between the cytochrome test and the phagocytosis (fluorescent **beads**) test or phagocytosing-killing (DCFH-DA) test by **flow cytometry** techniques. It is suggested that this might be caused by the fact that for technical reasons the two tests employed different stimulants and different substances to be phagocytosed. The simple **flow cytometry** procedure is now in widespread use as a PMN function test rather than the cytochrome C or nitroblue tetrazolium (NBT) **dye** reduction tests, and caution should be exercised in comparing and interpreting test results reported from separate laboratories. Because results using these two tests do not necessarily agree with each other, it seems preferable for PMN function testing to employ a combination of two or more approaches relying on different assay principles.

L20 ANSWER 48 OF 59 EMBASE
ACCESSION NUMBER: 93343003 EMBASE
DOCUMENT NUMBER: 1993343003
TITLE: Mouse thymic dendritic cell subpopulations.
AUTHOR: Ardavin C.; Wu L.; Ferrero I.; Shortman K.
CORPORATE SOURCE: Department of Cell Biology, Faculty of Biology, Complutense University,28040 Madrid, Spain
SOURCE: Immunology Letters, (1993) 38/1 (19-25).

ISSN: 0165-2478 CODEN: IMLED6
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Mouse thymic dendritic cells (DC) have been isolated after collagenase digestion, selection of the low-density cell fraction, then depletion of T-lineage cells and other non-DC by treatment with specific monoclonal antibodies (mAb) and removal with anti-Ig-**coated** magnetic **beads**. The resulting DC preparation represented 0.1-0.2% of total thymic cells and contained 70-80% DC. **Flow cytometry** analysis of MHC class II (MHC II) expression by DC showed that 40% of DC expressed intermediate levels of MHC II, and 60% expressed high levels of this marker. Moreover, immunofluorescent 2-colour **staining** allowed the characterization of two clearly **distinguishable** DC **subpopulations**: MHC II(inter) DC were CD45(hi), CD44(hi), HSA(hi), whereas MHC II(hi) DC were CD45(lo), CD44(lo), HSA(lo). These results are discussed with regard to the functional significance of MHC II(inter) and MHC II(hi) DC subpopulations in the mouse thymus.

L20 ANSWER 49 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 92258900 EMBASE
DOCUMENT NUMBER: 1992258900
TITLE: Serological detection of Helicobacter pylori by a flow **microsphere** immunofluorescence assay.
AUTHOR: Best L.M.; Van Zanten S.J.O.V.; Bezanson G.S.; Haldane D.J.M.; Malatjalian D.A.
CORPORATE SOURCE: Department of Microbiology, Victoria General Hospital, Dalhousie University, 5788 University Avenue,Halifax, NS B3H 1V8, Canada
SOURCE: Journal of Clinical Microbiology, (1992) 30/9 (2311-2317).
ISSN: 0095-1137 CODEN: JCMIDW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **A flow cytometric** immunofluorescence assay (FMIA) for the detection of immunoglobulin G antibodies to Helicobacter pylori was developed. A multicomponent antigen was prepared and used to **coat** carboxylated polystyrene **microspheres** for reaction with patient sera followed by

fluorescein isothiocyanate-**labelled** goat anti-human immunoglobulin G. The reacted **microspheres** were collected with a **flow cytometer**, and fluorescence was quantitated relative to the cutoff value provided by pooled sera from patients in whom H. pylori could not be demonstrated by culture or histology. Serum samples from 28 H. pylori-positive patients and 27 H. pylori-negative patients were tested by FMIA. Additionally, an in-house enzyme-linked immunosorbent assay (ELISA) employing the same antigen preparation and a commercially available ELISA were used to **assay** the patient **population**. Both the FMIA and in-house ELISA were 100% sensitive and 89% specific with positive and negative predictive values of 90 and 100% and no equivocal results. The commercial ELISA was 96% sensitive and 89% specific with positive and negative predictive values of 90 and 96% and five equivocal results. FMIA provides a rapid, inexpensive, and easily performed means for serodiagnosis of H. pylori.

L20 ANSWER 50 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92365878 EMBASE
DOCUMENT NUMBER: 1992365878
TITLE: Activation of platelets in blood perfusing angioplasty-damaged coronary arteries: **Flow cytometric** detection.
AUTHOR: Scharf R.E.; Tomer A.; Marzec U.M.; Teirstein P.S.; Ruggeri Z.M.; Harker L.A.
CORPORATE SOURCE: Division of Hematology/Oncology, Emory University School of Medicine, PO Drawer AR,Atlanta, GA 30322, United States
SOURCE: Arteriosclerosis and Thrombosis, (1992) 12/12 (1475-1487).
ISSN: 1049-8834 CODEN: ARTTE5
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 005 General Pathology and Pathological Anatomy
018 Cardiovascular Diseases and Cardiovascular Surgery
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Fluorescence-activated **flow cytometry** has been used to investigate platelet activation in blood flowing through atherosclerotic coronary arteries after sustaining mechanical damage induced by percutaneous transluminal angioplasty (PTCA). For **flow cytometry**, platelets and platelet- derived **microparticles** were identified by biotinylated anti-glycoprotein (GP) Ib monoclonal antibody (mAb) and a fluorophore, phycoerythrin-streptavidin. Activated platelets were detected by using a panel of fluoresceinated mAbs specific for activation-dependent platelet epitopes, including 1) activated

GPIIb-IIIa complex (PAC1); 2) fibrinogen bound to platelet GPIIb-IIIa (9F9); 3) ligand-induced binding sites on GPIIIa (anti-LIBS1); and 4) P-selectin, an .alpha.-granule membrane protein expressed on the platelet surface after secretion (S12). The binding of antibodies to platelets was **determined** in blood that was **sampled** continuously via heparin-**coated** catheters from the coronary sinus in 1) patients before, during, and for 30 minutes after PTCA and 2) control patients undergoing coronary angiography without PTCA. Platelets in coronary sinus blood showed significant binding of mAbs that specifically detect activation epitopes associated with the GPIIb-IIIa complex (PAC1, anti- LIBS1, and 9F9) during and for 30 minutes after angioplasty in four of the five patients. The relative proportion of platelets positive for PAC1 and anti-LIBS1 increased from baseline values of 2.0.+-.0.3% (mean.+-.SD) and 2.0.+-.0.5% to 18.+-.14% and 28.+-.14%, respectively, during PTCA or 30 minutes after PTCA ($p<0.01$ in both cases). Binding with 9F9 was less prominent. The expression of P-selectin was detected in one of the five patients. By contrast, activation-specific mAbs failed to bind detectably with platelets obtained from 1) the peripheral blood during coronary angiography in eight patients or 2) coronary sinus blood obtained via catheter throughout control catheterization procedures in three patients or before PTCA in five. We conclude that circulating platelets become activated while flowing through PTCA-damaged stenotic coronary arteries and that this process of platelet activation is readily demonstrated by measuring the expression of activation- specific membrane GP epitopes by **flow cytometric** analysis.

L20 ANSWER 51 OF 59 MEDLINE
ACCESSION NUMBER: 92389781 MEDLINE
DOCUMENT NUMBER: 92389781 PubMed ID: 1518408
TITLE: **Flow cytometric** micronucleus test with mouse peripheral erythrocytes.
AUTHOR: Hayashi M; Norppa H; Sofuni T; Ishidate M Jr
CORPORATE SOURCE: Division of Genetics and Mutagenesis, National Institute of Hygienic Sciences, Tokyo, Japan.
SOURCE: MUTAGENESIS, (1992 Jul) 7 (4) 257-64.
Journal code: MUG; 8707812. ISSN: 0267-8357.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199210
ENTRY DATE: Entered STN: 19921023
Last Updated on STN: 20000303
Entered Medline: 19921008
AB **Flow cytometric** (FC) analysis was applied to

micronucleus test with mouse peripheral blood erythrocytes. The method is based on the measurement of peak fluorescence (PFL) of **sphered** glutaraldehyde-fixed erythrocytes before and after **staining** with 4',6-diamidino-2-phenylindole (DAPI), in an EPICS V **flow cytometer**. The frequency of micronucleated erythrocytes (MNEs) is calculated by a computer program comparing PFL data obtained with and without DAPI. To evaluate the method, male ddY mice were treated with 6-mercaptopurine and benzene and blood was collected from tail vein at intervals of 4-7 days. Both microscopic and FC analysis showed a steady increase in the incidence of MNEs, reaching a plateau when about a month had passed from the start of the treatments. The effects of benzo[a]pyrene, mitomycin C,N-ethyl-N-nitrosourea, bromodichloromethane and potassium chromate (VI) were also studied with both the manual and FC **assay** in **samples** collected a week after five weekly treatments. The percentages of MNEs obtained manually and by the FC measurements showed good correlation, the former three chemicals being positive and the latter two negative or, in the FC analysis, difficult to classify. Because of the high number of cells examined (50,000/animal), the FC analysis was probably more sensitive than the manual method where only 2000 cells were scored per animal. This was further suggested by (i) steady time responses, also for individual animals, in the FC results on 6-mercaptopurine and benzene, (ii) overall reduced inter-individual variation in the FC measurements, and (iii) detection of MNE induction by mitomycin C at a lower dose level with the FC than the manual analysis.(ABSTRACT TRUNCATED AT 250 WORDS)

L20 ANSWER 52 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92245322 EMBASE
DOCUMENT NUMBER: 1992245322
TITLE: Analyzing the distribution of cells expressing mRNA for T cell receptor .gamma. and .delta. chains in a virus-induced inflammatory process.
AUTHOR: Allan W.; Carding S.R.; Eichelberger M.; Doherty P.C.
CORPORATE SOURCE: Department of Immunology, St. Jude Children's Research Hosp.,Memphis, TN 38105, United States
SOURCE: Cellular Immunology, (1992) 143/1 (55-65).
ISSN: 0008-8749 CODEN: CLIMB8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Acute inflammatory processes are extremely complex, containing sets of activated cells that may be difficult to categorize. The

interface between two methodologies for characterizing the involvement of .gamma..delta. T cells, in situ hybridization to detect T cell receptor (TCR) mRNA and **flow cytometric** analysis of surface TCR expression, is utilized here to study the pneumonia caused by intranasal (i.n.) infection of mice with influenza A viruses. Substantial numbers of cells expressing mRNA for the .gamma. and .delta. TCR chains are present in bronchoalveolar lavage (BAL) populations obtained either late in the course of primary infection with an H3N2 virus or within a few days of secondary challenge with an H1N1 virus. The majority of the .gamma..delta. TCR mRNA+ cells **detected** in FACS-**separated** BAL **populations** partition to the Thy1+ .gamma..delta. TCR+ subset, while relatively few (<10%) C.delta. mRNA transcripts are found in cells that phagocytose latex **particles**. However, an additional set of .gamma..delta. TCR mRNA+ cells is also located in a high side scatter (H-SSC) population, which **stains** nonspecifically with monoclonal antibodies (mAbs) and is normally gated out in the process of **flow cytometric** analysis. This H-SSC population tends to be enriched for cells expressing C.gamma.1/2 rather than C.gamma.4 mRNA. While some .gamma..delta. TCR+ lymphocytes can be demonstrated by in vitro stimulation of the CD3.epsilon.+ subset within this H-SSC population, the majority of the .gamma..delta. T cell precursors that can be expanded in culture demonstrate a low side scatter (L-SSC) profile more characteristic of normal T lymphocytes. The possibility that subsets of activated, granular (H-SSC) .alpha..beta. TCR+ and C.gamma.1/2 mRNA+ .gamma..delta. T cells are being missed when conventional FACS analysis is used to study this viral pneumonia is discussed.

L20 ANSWER 53 OF 59 MEDLINE
ACCESSION NUMBER: 93055241 MEDLINE
DOCUMENT NUMBER: 93055241 PubMed ID: 1331158
TITLE: Quantitative **flow cytometry** of mouse mammary tumor virus envelope glycoprotein (gp52): alternative measures of hormone-mediated change in a viral cell surface antigen.
AUTHOR: Ritzi E M
CORPORATE SOURCE: Department of Microbiology, Texas Tech University Health Sciences Center, Lubbock 79430.
SOURCE: JOURNAL OF VIROLOGICAL METHODS, (1992 Oct) 40 (1) 11-30.
Journal code: HQR; 8005839. ISSN: 0166-0934.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199211

Searcher : Shears 308-4994

ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19970203
Entered Medline: 19921127

AB An immunofluorescence procedure with C3H mouse mammary tumor cells (Mm5mt/cl) has incorporated **flow cytometry** to provide a fluorescence-based measurement of changes in the mouse mammary tumor virus (MMTV) cell surface glycoprotein (gp52). A comparison of mean channel fluorescence intensity (delta mean) of cell populations **stained** with immune sera and NRS permitted a gp52-specific signal to be measured for controls and cells treated with 10(-6) M dexamethasone (Dex). Three different methods have been developed to quantitatively compare gp52-related fluorescence on control and hormone-treated cells. First, delta mean, measured as a gp52-specific difference in channel number was 169-209 for control cells and 299-341 for Dex-treated cells. These fluorescence measurements with 4 different sera demonstrated gp52-specific increases due to Dex treatment of 141, 130, 143, and 115 channels. A second method of gp52 quantitation **determined** the percentage shift in **staining populations** over NRS and specified channel intensity markers. Dex treatment resulted in a 6.9 to 32.4% shift over channel 508 (NRS marker) and a more marked shift of 45.5 to 49.2% over channel 676 (control cell marker). A third methodology utilized fluorescein **bead** standards to calculate molecules of equivalent soluble fluorescein (MESF). These MESF determinations permitted hormonal effects to be measured as fold increases over controls. Dex induction of gp52 for C3H and GR mammary tumor cells ranged from 1.5 to 9.1 fold increases. Alternative steroid treatments and antibody directed against the internal cytoplasmic MMTV P27 provided negative controls for measurements of changing gp52 levels.

L20 ANSWER 54 OF 59 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 91173627 EMBASE
DOCUMENT NUMBER: 1991173627
TITLE: The effect of improvements in cytometer sensitivity on the detection of CD5-positive B cells with dim fluorescence.
AUTHOR: Givan A.L.; Calvert J.E.; Shenton B.K.
CORPORATE SOURCE: Department of Surgery, Newcastle University, Medical School,Newcastle upon Tyne NE2 4HH, United Kingdom
SOURCE: Cytometry, (1991) 12/4 (360-365).
ISSN: 0196-4763 CODEN: CYTODQ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 025 Hematology
026 Immunology, Serology and Transplantation
LANGUAGE: English

SUMMARY LANGUAGE: English

AB When antigen density on the surface of a cell population is low and variable, the percentage of that **population determined** to express the antigen (i.e., to be positively **stained**) depends directly on the sensitivity of the **flow cytometer** for resolving **particles** which are dimly fluorescent from those which are unstained. In this study, the sensitivity of a commercial **flow cytometer** has been improved by changes in the photomultiplier tube, the fluorescence filter, and the amount of stray light entering the fluorescence channel. In a model system with human lymphocytes, modifications to these factors increased the percent of the B-lymphocyte population found to express the CD5 antigen.

L20 ANSWER 55 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1989-356576 [48] WPIDS
DOC. NO. NON-CPI: N1989-271106
DOC. NO. CPI: C1989-158164
TITLE: **Flow cytometric assay** for **analyte**(s) in aq. **sample** - utilising pairs of mono disperse **particles** carrying specific binding partner, with increased dynamic range.
DERWENT CLASS: B04 J04 S03
INVENTOR(S): LINDMO, T
PATENT ASSIGNEE(S): (SINV-N) SINVENT AS; (DYNA-N) DYNAL AS; (HOLM-I) HOLMES M J; (LIND-I) LINDMO T
COUNTRY COUNT: 16
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8911101	A	19891116	(198948)*	EN	31
RW: AT BE CH DE FR GB IT LU NL SE					
W: AU GB JP NO US					
NO 8802067	A	19891204	(199003)		
AU 8935658	A	19891129	(199007)		
EP 413741	A	19910227	(199109)		
R: AT BE CH DE FR GB IT LI LU NL SE					
JP 03504276	W	19910919	(199144)		
CA 1335238	C	19950418	(199523)		
EP 413741	B1	19950830	(199539)	EN	17
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 68924064	E	19951005	(199545)		
US 5585241	A	19961217	(199705)		13
JP 2742953	B2	19980422	(199821)		10

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 8911101	A		WO 1989-GB506	19890510
EP 413741	A		EP 1989-905720	19890510
JP 03504276	W		JP 1989-505599	19890310
CA 1335238	C		CA 1989-599225	19890511
EP 413741	B1		EP 1989-905720	19890510
			WO 1989-GB506	19890510
DE 68924064	E		DE 1989-624064	19890510
			EP 1989-905720	19890510
			WO 1989-GB506	19890510
US 5585241	A	Cont of	US 1990-602269	19901210
		Cont of	US 1992-924397	19920803
			US 1995-457158	19950601
JP 2742953	B2		JP 1989-505599	19890510
			WO 1989-GB506	19890510

FILING DETAILS:

PATENT NO	KIND		PATENT NO
EP 413741	B1	Based on	WO 8911101
DE 68924064	E	Based on	EP 413741
		Based on	WO 8911101
JP 2742953	B2	Previous Publ.	JP 03504276
		Based on	WO 8911101

PRIORITY APPLN. INFO: NO 1988-2067 19880511

AN 1989-356576 [48] WPIDS

AB WO 8911101 A UPAB: 19930923

Assay method for one or more **analytes** in an aq-sample is claimed. For each **analyte** (I) to be assayed, a pair of different monodisperse **particle** types is used, each **particle** carrying a binding partner with the same specificity but different binding affinity for (I). The pair of **particle** types which has reacted with (I) and become **labelled** by a **labelled** ligand also present in the reaction mixt., is distinguished by **flow cytometry** from each other and from pairs of **particle** types, which have reacted with each other **analyte** (I') to be assayed.

Specifically, (I) and binding partner pairs may be (a) antigen and specific antibody; (b) hormone and hormone receptor; (c) hapten and anti-hapten; (d) polynucleotide and complementary polynucleotide; (e) polynucleotide and polynucleotide binding protein; (f) biotin and avidin or streptaridin; (g) enzyme and enzyme cofactor; or (h) lectin and specific carbohydrate.

USE/ADVANTAGE - The method is esp. suited to sandwich type assays. It may also be used to determine binding affinity and binding kinetics of substances attached onto one type of **particle**. The method shows improved sensitivity of related techniques; it provides additional reliability as it uses a double standard curve, one form each **particle** type; and an increased dynamic range, e.g. with small **particles** used for the high-affinity antibody and larger **particles** for the antibody of lower affinity. The method exploits the advantages of **flow cytometric** reading in that sepn. of free and bound **reagents** is not required. It also aoivds the Hook-effect.

1/7

ABEQ EP 413741 B UPAB: 19951004

A method of assay of one or more **analytes** in an aqueous sample wherein for each **analyte** to be assayed monodisperse **particles** carrying a specific binding partner for that **analyte** are used to bind the said **analyte** in the sample and a **labelled** ligand is used to indicate the amount of the bound **analyte**, the amount of **labelled** ligand bound to the **particles** being determined by a **flow cytometer**, characterised in that for each **analyte** to be assayed a pair of different **particle** types is used, the **particles** of each of the two **particle** types of the pair carrying a binding partner having the same specificity but having a different binding affinity for the **analyte**, the pair of **particle** types which has reacted with each **analyte** to be assayed and become **labelled** by a **labelled** ligand being distinguishable by the **flow cytometer** from each other and from any other pairs of **particle** types which have reacted with any other **analyte** or **analytes** to be assayed.

Dwg.1/7

ABEQ US 5585241 A UPAB: 19970129

In a method for the **flow cytometric** assay of an **analyte** in an aqueous sample using monodisperse **particles** carrying a specific binding partner therefor, said **analyte** and binding partner being selected from the group consisting of (a) antigen and specific antibody, (b) hormone and hormone receptor, (c) hapten and antihapten, (d) polynucleotide and polynucleotide binding protein, (e) biotin and avidin or streptavidin, and (f) lectin and specific carbohydrate, and said method comprising the steps of adding to the aqueous sample a predetermined amount of said **particles** and a predetermined amount of a **labelled** ligand having affinity for the **analyte** or the binding partner and detecting and quantifying the resulting **labelled** ligand-carrying **particles**

by means of a **flow cytometer**, the improvement consisting of using a pair of a different **particle** types which are distinguishable from each other by the **flow cytometer** and which respectively carry binding partners having the same specificity but different binding affinity for the **analyte** and independently but simultaneously detecting and quantifying the two types of **labelled** ligand-carrying **particles** by means of the **flow cytometer** and determining the **analyte** concentration from the thus-obtained two measurement values by reference to a double standard calibration curve; said double standard calibration curve having one curve which quantitatively shows the amount of **label** bound to one of the pairs of **particle** types as a function of **analyte** concentration in the sample and another curve which quantitatively shows the amount of **label** bound to the other **particle** type as a function of **analyte** concentration in the sample.
Dwg.0/7

L20 ANSWER 56 OF 59 MEDLINE
ACCESSION NUMBER: 89327474 MEDLINE
DOCUMENT NUMBER: 89327474 PubMed ID: 2502559
TITLE: Rapid **flow cytometric** bacterial detection and determination of susceptibility to amikacin in body fluids and exudates.
AUTHOR: Cohen C Y; Sahar E
CORPORATE SOURCE: Sackler School of Medicine, Tel-Aviv University, Israel.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1989 Jun) 27 (6) 1250-6.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198909
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19900309
Entered Medline: 19890901

AB A **flow cytometry**-based method for rapid and quantitative **detection** of bacteria in various clinical **specimens** and for rapid **determination** of antibiotic effect is described. Achieving such a measurement with high sensitivity required discrimination between bacteria and other **particles** which were often present in clinical samples in high concentrations. This discrimination was facilitated by detecting the bacterial characteristic light scatter and fluorescence signals following **staining**, e.g., with the

fluorescent nucleic acid-binding **dye** ethidium bromide, as well as by measuring bacterial proliferation during short time intervals. Antibiotic susceptibility was measured by observing the inhibition of such proliferation. The method was applied to 43 clinical specimens from various sources, such as wound exudates, bile, serous cavity fluids, and bronchial lavage. Bacterial detection, achieved in less than 2 h, agreed with results of conventional methods with a sensitivity of 74% and a specificity of 88%. Susceptibility to amikacin was detected in 1 h in 92% of 13 positive specimens.

L20 ANSWER 57 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1986-240616 [37] WPIDS
CROSS REFERENCE: 1989-300360 [41]; 1989-347915 [47]; 1990-156075 [20]; 1991-036853 [05]; 1991-238140 [32]; 1992-016179 [02]; 1992-056351 [07]; 1992-079433 [10]; 1994-166592 [20]; 1995-060333 [08]
DOC. NO. NON-CPI: N1986-179803
DOC. NO. CPI: C1986-103422
TITLE: Uniformly sized **microbeads** of hydrophobic polymer - coupled to fluorescent **dye**, useful as standards for calibrating microscopes and **flow cytometer**(s).
DERWENT CLASS: A89 J04 S03
INVENTOR(S): SCHWARTZ, A
PATENT ASSIGNEE(S): (FLOW-N) FLOW CYTOMETRY STANDARDS CORP
COUNTRY COUNT: 4
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
GB 2172104	A	19860910	(198637)*		15
JP 61228349	A	19861011	(198647)		
US 4714682	A	19871222	(198801)		
US 4767206	A	19880830	(198837)		
US 4774189	A	19880927	(198841)		
GB 2172104	B	19881109	(198845)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
GB 2172104	A	GB 1985-531043	19851217
JP 61228349	A	JP 1985-291827	19851224
US 4714682	A	US 1987-33625	19870403
US 4767206	A	US 1984-685464	19841224
US 4774189	A	US 1985-805654	19851211

PRIORITY APPLN. INFO: US 1984-685464 19841224; US 1985-805654 19851211; US 1987-33625 19870403; US 1987-109214 19871015; US 1987-128786 19871204

AN 1986-240616 [37] WPIDS

CR 1989-300360 [41]; 1989-347915 [47]; 1990-156075 [20]; 1991-036853 [05]; 1991-238140 [32]; 1992-016179 [02]; 1992-056351 [07]; 1992-079433 [10]; 1994-166592 [20]; 1995-060333 [08]

AB GB 2172104 A UPAB: 19950306

Microbead product (A) comprises uniformly-sized, **spherical particles** of hydrophobic polymer (I), approx. the same size as cells, covalently bonded, at the **particle** surface, to a fluorescent **dye** (II). When suspended in a medium, the **particles** have the same fluorescent spectrum as a soln. of (II). Pref. (A) have diam. 1-100 microns with fluorescence intensity 100-10 million equiv. soluble molecules of (II) per **microbead**, and are stable (with respect to size and fluorescence intensity) in the suspension medium.

(I) is derived from styree, vinyl toluene and (meth)acrylate esters plus glycidyl methacrylate (III), allyl glycidyl ether or other epoxy-contg. **cpds**. esp. 95% methyl methacrylate-5% (III). The **dye** can be phycoerythrin, phycocyanin or allophycocyanin (attached by direct reaction of NH2 gps. with epoxy gps. in the **particle**), or a biotin-conjugate of these **cpds**. (attached via avidin molecules coupled to the **particle**). Alternatively, the epoxy gps. are reacted with a diamine and the aminated surface reacted with fluorescein isothiocyanate or Texas Red.

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Dwg.0/10

ABEQ GB 2172104 B UPAB: 19930922

Microbead product (A) comprises uniformly-sized, **spherical particles** of hydrophobic polymer (I), approx. the same size as cells, covalently bonded, at the **particle** surface, to a fluorescent **dye** (II). When suspended in a medium, the **particles** have the same fluorescent spectrum as a soln. of (II). Pref. (A) have diam. 1-100 microns with fluorescence intensity 100-10 million equiv. soluble molecules of (II) per **microbead**, and are stable (with respect to size and fluorescence intensity) in the suspension medium.

(I) is derived from styrene, vinyl toluene and (meth)acrylate esters plus glycidyl methacrylate (III), allyl glycidyl ether or other epoxy-contg. **cpds**. esp. 95% methyl methacrylate-5% (III). The **dye** can be phycoerythrin, phycocyanin or allophycocyanin (attached by direct reaction of NH2 gps. with epoxy gps. in the **particle**), or a biotin-conjugate of these

cpds. (attached via avidin molecules coupled to the **particle**). Alternatively, the epoxy gps. are reacted with a diamine and the aminated surface reacted with fluorescein isothiocyanate or Texas Red.

0/10

ABEQ US 4714682 A UPAB: 19930922

Calibration **microbead** standard is produced by (a) homogenising highly insoluble (less than 0.001 g/l.) **cpd**. (I) of mol. wt. less than 1000 in a surfactant soln. to obtain a first homogenate; (b) swelling small seed **microbeads**, 0.1-3 micron in dia. with the first homogenate; (c) homogenising an aq. surfactant soln. contg. a large stabilising alkaline halide salt (II) with a mixt. of oil soluble polymerisable monomers (III) in which is dissolved 0.5-5 wt.% oil soluble initiator (IV) to obtain a second homogenate, (d) swelling the seed **microbeads** with the second homogenate; and (e) placing the **microbeads** under conditions to cause the initiator to polymerise the monomers in the swollen **microbeads**.

Pref. (I) is 1-chlorododecane; (II) is KCl; (III) are methyl and glycidyl methacrylate, and (IV) is benzoyl peroxide.

USE/ADVANTAGE - Aggregation is minimised and yield is maximised. Used for calibrating a **flow cytometer** or fluorescent microscope.

ABEQ US 4767206 A UPAB: 19930922

Flow cytometer calibration reference standard is established using (a) first batch of standard highly uniformly sized **microbeads** of hydrophobic polymeric material 3-15 micron in size, with fluorescent **dye** material covalently bound to the surface and (b) a second batch of primary **microbeads** comprising hydrophilic polymeric material with fluorescent **dye** copolymerised within the **microbeads**.

Excitation emission spectra of the **microbeads** of both batches are identical to each other to the same free **dye**, when suspended in the same soln. and to a **particle** sample of cells incorporating the **dye**. Having established this, (b) is calibrated against a **sample** of the free **dye** to **determine** the equiv. soluble fluorescent molecules per **microbead**. A calibration curve for (b) representing equiv. sol. fluorescent molecules are relative fluorescent channel of a **flow cytometer** is developed. The fluorescent intensities of (a) are measured with a **flow cytometer** to determine its relative fluorescent channel. Then, the number of equiv. sol. fluorescent molecules per **microbeads** in (a) is determined to establish (a) as a standard reference.

ABEQ US 4774189 A UPAB: 19930922

Microbead prod. (I) comprises (a) a highly-uniform size **spherical** body having a dia. of 1-100 microns and formed of

hydrophobic polymeric material comprising a polymerisation prod. of an ethylenically unsatd. monomer having an epoxy functionality which, after polymerisation, is at the surface of (a); and (b) a prim. amine-contg. fluorescent **dye** material (II) directly covalently bonded to the surface of the body material via the epoxy functional gps. and in such manner that, when (I) are suspended in a media, (I) exhibit a fluorescent spectra equiv. to that of the material (II) dissolved in the same media.

Pref. the hydrophobic polymeric material comprises a copolymer of 95% methyl methacrylate and 5% glycidyl methacrylate.

USE - (I) is useful as a standard for aligning and calibrating fluorescent microscopes and **flow cytometers**.

L20 ANSWER 58 OF 59 BIOSIS COPYRIGHT 2001 BIOSIS
ACCESSION NUMBER: 1987:85592 BIOSIS
DOCUMENT NUMBER: BA83:44170
TITLE: EFFECT OF HYPOTHALAMIC LESIONS ON LYMPHOCYTE SUBSETS IN MICE.
AUTHOR(S): KATAYAMA M
CORPORATE SOURCE: DEP. NEUROSURG., KURUME UNIV. SCH. MED.
SOURCE: J KURUME MED ASSOC, (1986) 49 (6), 446-457.
CODEN: KIZAAL. ISSN: 0368-5810.
FILE SEGMENT: BA; OLD
LANGUAGE: Japanese

AB The purpose of this study is to analyze the effects of hypothalamic lesions on lymphocyte subsets in order to better understand the roles of the central nervous system in the immune response. Four-week-old C57BL/6 mice were used in this study. Bilateral electrolytic lesions were produced in the anterior (A group), middle (M group) or posterior part (P group) of the hypothalamus using Narishige's stereotaxic instrument and Radionics's lesion generator. Age-matched, non-operated mice (**N group**), and sham-operated mice with cerebral **hemisphere** lesions through the electrodes but without application of current (S group) were used as controls. Peripheral blood lymphocyte subsets were analyzed in each group using monoclonal antibodies to T cell antigens; Thy 1, 2, Lyt 1, Lyt 2 and an antibody for SmIg with a laser **flow cytometry** system once every week for four weeks after operation. The Lyt 1/Lyt 2 ratio, could be used as a parameter of the immunoregulative condition, was also examined. The ratio of the control group showed a transient low value at first week, but returned to the initial level thereafter. In the A and P groups, the values decreased shortly after the operation, and showed continually low value until fourth week. SmIg cells representing B lymphocytes also increased. On the other hand, in the M group, although the value of the ratio and the number of SmIg cells decreased remarkably, that of the ratio returned to control levels there after. The present study shows that the hypothalamus contains

at least two regions influencing the host immune response. The anterior and posterior parts of the hypothalamus, seem to play a role in the immune modulation of both helper and suppressor T lymphocytes. The remaining middle part of the hypothalamus is considered to play a control mechanism in the immune regulation of T and B lymphocytes.

L20 ANSWER 59 OF 59 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1983-25545K [11] WPIDS
DOC. NO. NON-CPI: N1983-046423
DOC. NO. CPI: C1983-024995
TITLE: Electro-optical determination of red blood cell vol. - with blood sample treated with sphering agent and protein for stability.

DERWENT CLASS: B04 J04 S03
INVENTOR(S): KIM, Y R; ORNSTEIN, L
PATENT ASSIGNEE(S): (TECD) TECHNICON INSTR CORP
COUNTRY COUNT: 16
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 73554	A	19830309	(198311)*	EN	15
R: BE CH DE FR GB IT LI NL SE					
JP 58006468	A	19830114	(198311)		
US 4412004	A	19831025	(198345)		
CA 1170553	A	19840710	(198432)		
EP 73554	B	19850313	(198511)	EN	
R: BE CH DE FR GB IT LI NL SE					
DE 3262531	G	19850418	(198517)		
AU 8436033	A	19850530	(198529)		
JP 03046784	B	19910717	(199132)		
US 5045472	A	19910903	(199138)		6
JP 06050970	A	19940225	(199413)		5
JP 07069324	B2	19950726	(199534)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 73554	A	EP 1982-302524	19820518
JP 03046784	B	JP 1982-63257	19820417
US 5045472	A	US 1990-537887	19900614
JP 06050970	A	JP 1990-417972	19901219
JP 07069324	B2	JP 1990-417972	19820417

FILING DETAILS:

PATENT NO	KIND	PATENT NO
JP 07069324	B2 Based on	JP 06050970

PRIORITY APPLN. INFO: US 1981-277539 19810626
AN 1983-25545K [11] WPIDS
AB EP 73554 A UPAB: 19930925
Mammalian whole blood is treated to give a **sample** for electro-optical **determn**. of red blood cell vol. and it involves treating the whole blood with a first isotonic soln. contg. a sphering agent. Then an aliquot of the resulting mixt. is treated with a second isotonic soln. contg. protein and sphering agent. The wt. ratio of protein to sphering agent in the aliquot and in the final sample is 20:1-70:1; and the concn. of sphering agent in the final sample is 2-10 mg/100 ml.

Prepn. of stable, **sphered** and fixed mammalian red blood cells as calibration **particles** for **flow cytometry** comprises (a) combination of an anticoagulated whole blood sample with an isotonic soln. contg. sphering agent, followed by (b) treatment of the mixt. with a fixing agent soln. The prod. of step (a) has a wt. ratio of endogenous protein to sphering agent of 20:1-70:1 and a final concn. of sphering agent of 2-10 mg/100 ml.

Prolonged stability of the **sphered** red blood cells is achieved by control of the concn. of sphering agent and its ratio to protein. The protein ensures shaped consistency during processing as well as minimising change during handling etc. The procedures are used in diagnostic examination of red blood cell vol. etc.

ABEQ EP 73554 B UPAB: 19930925
Mammalian whole blood is treated to give a **sample** for electro-optical **determn**. of red blood cell vol. and it involves treating the whole blood with a first isotonic soln. contg. a sphering agent. Then an aliquot of the resulting mixt. is treated with a second isotonic soln. contg. protein and sphering agent. The wt. ratio of protein to sphering agent in the aliquot and in the final sample is 20:1-70:1; and the concn. of sphering agent in the final sample is 2-10 mg/100 ml.

Prepn. of stable, **sphered** and fixed mammalian red blood cells as calibration **particles** for **flow cytometry** comprises (a) combination of an anticoagulated whole blood sample with an isotonic soln. contg. sphering agent, followed by (b) treatment of the mixt. with a fixing agent soln. The prod. of step (a) has a wt. ratio of endogenous protein to sphering agent of 20:1-70:1 and a final concn. of sphering agent of 2-10 mg/100 ml.

Prolonged stability of the **sphered** red blood cells is achieved by control of the concn. of sphering agent and its ratio to protein. The protein ensures shaped consistency during processing as

well as minimising change during handling etc. The procedures are used in diagnostic examination of red blood cell vol. etc.

ABEQ US 5045472 A UPAB: 19930925

Compsn. for use in a cytometer, comprises: (a) an anticoagulated whole blood sample aliquot; and (b) a **reagent** mixt. of (i) an isotonic aq. soln.; (ii) a sphering agent; and (iii) a protein which reversibly binds the sphering agent (ii) and (iii) are present in wt. ratio of protein to sphering agent of 20:1 to 70:1. The sphering agent has a final concn. between 2mg per 100ml-10mg per 100ml in the **reagent** mixt.

Pref. protein is a serum albumin selected from bovine, human and egg, which is endogenous in whole blood sample. Pref sphering agent is detergent, phospholipid or fatty acid. The **reagent** mixt. may also contain a fixing agent.

USE - For more accurate and precise electroptical method for measuring erythrocyte volumes individually and as an average. @@

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